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# CANCER RESEARCH

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CANCER RESEARCH
A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH
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# Some Influences of Hormones on the Growth and Persistence of Transplanted Testicular Tumors*

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(Received for publication April 18, 1945)

The influence of steroid hormones upon the genesis of tumors and upon the rate or frequency of growth of transplanted or spontaneous tumors has been studied extensively in experimental animals. Either intrinsic or extrinsic hormones are required in the genesis of mammary, testicular, and hypophyseal tumors in mice, for example. In addition, genetic or transmitted factors determine in part the carcinogenic response of mice treated with estrogens (5, 12). The humoral environment also regulates the growth of some transplanted mammary tumors; in rats, estrogens in general stimulate their growth (6, 7, 13).

Testosterone decreased the growth rate of some mammary tumors and the amount of glandular tissue that they contained (7, 14). Progesterone also inhibited the growth of spontaneous or transplanted mammary adenofibromas in rats, although large amounts of progesterone could not counteract the stimulating effects of estrogen (8, 15). In animals given progesterone there was not only a reduced growth of the tumors; some regressed or disappeared (15).

The practical aspect of the problems concerned with the influences of humoral factors on the progressive growth of neoplasia has been demonstrated in patients with prostatic tumors, many of whom are symptomatically improved by orchidectomy or the administration of stilbestrol (11). The assumption might be made that certain neoplasms are completely autonomous within their original environment but depend upon a specific humoral contribution to this environment. Also, specific hormones may either inhibit or augment the growth of different tumors or may oppose or facilitate the production of some substance necessary for their progressive growth.

Some neoplasms grow more rapidly in male than in female mice, or almost selectively in male mice (17). Interstitial cell tumors of the testis arising in estrogen-treated mice have grown subsequent to transplantation when placed in hosts that received estrogen (1, 2, 3, 10). The present experiments were undertaken to determine some of the factors that might maintain an environment compatible with the growth of these tumors.

## MATERIALS AND METHODS

The transplanted testicular interstitial cell tumors used in this investigation arose originally in a mouse of the A strain [1] and in a hybrid mouse obtained by mating a female of the A strain to a male of the C3H strain. The original growths and all subsequent generations of transplanted tumors were inoculated by inserting small grafts subcutaneously along the lateral body wall through a thirteen-gauge hypodermic needle fitted with a plunger. The hosts were mice of the (a) A, C3H, or CBA strain; (b) hybrid mice obtained by mating mice of the A strain with mice of the C3H, CBA, C57, or JK strains; or (c) other hybrid crosses in which the A strain was not represented.

All mice were maintained on Purina fox chow and water in an air conditioned room. Those that received the implants were divided into three groups. Mice of one group received no treatment subsequent to implantation, but were observed throughout their life span or until all the mice in the other groups were dead (Group 1, Tables I and II). A second

* This investigation has been supported by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The Anna Fuller Fund.

[1] The mice used in this investigation were obtained from or were the progeny of animals obtained from Dr. L. C. Strong.

TABLE I: FATE OF SUBCUTANEOUS TRANSPLANTS OF A TESTICULAR INTERSTITIAL CELL TUMOR (2ATE10) IN MICE OF THE A STRAIN, EITHER UNTREATED OR TREATED WITH STILBESTEROL

| Transplant generation * | Groups † | Treatment ‡ | No. of mice | Days of treatment | Days treatment was started subsequent to implantation | Days observed after stopping treatment | No. of mice with tumors | No. of mice without tumors |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | SS | 2 | 114-115 | — | — | 2 | — |
| 2 | 1 | — | 2 | 266 | — | — | — | 2 |
| | 2 | SS | 4 | 82-131 | — | — | 4 | — |
| | 2 | SS | 6 | 82-142 | — | 14-112 | 6 | — |
| | 2 | SC | 2 | 50-62 | — | — | 2 | — |
| 3 | 1 | — | 10 | 99-201 | — | — | — | 10 |
| | 2 | SS | 14 | 91-219 | — | — | 14 | — |
| | 2 | SC | 12 | 43-183 | — | — | 12 | — |
| | 2 | SS | 4 | 95 | — | 74-188 | 3 | 1 |
| | 3 | SC | 6 | 76-183 | 99 | — | 6 | — |
| 4 | 1 | — | 6 | 105-235 | — | — | — | 6 |
| | 2 | SC | 20 | 73-285 | — | — | 17 | 3 |
| 5 | 2 | SC | 5 | 61-109 | — | — | 5 | — |
| | 3 | SC | 4 | 153-222 | 107 | — | 4 | — |
| 6 | 1 | — | 5 | 205-268 | — | — | — | 5 |
| | 2 | SC | 10 | 73-160 | — | — | 10 | — |
| | 3 | SC | 7 | 35-205 | 51-204 | — | 6 | 1 |
| 7 | 1 | — | 1 | 133 | — | — | — | 1 |
| | 2 | SC | 5 | 77-153 | — | — | 3 | 2 |
| 8 | 2 | SC | 3 | 78-245 | — | — | 3 | — |
| | 2(3)§ | SC | 3 | 78 | 128 | — | 1 | 2 |
| | 3 | SC | 7 | 68-78 | 128-176 | — | 1 | 6 |
| 9 | 1 | — | 3 | 312 | — | — | — | 3 |
| | 2 | SC | 6 | 37-238 | — | — | 4 | 2 |
| | 3 | SC | 4 | 27-146 | 56 | — | 2 | 2 |

* The tumors were transplanted serially for 9 generations.

† Group 1, untreated mice; Group 2, received estrogen at the time of transplantation; Group 3, estrogen treatment started some time after transplantation.

‡ SS, weekly injections of 0.25 mgm. of stilbestrol; SC, pellets of stilbestrol 1 part and cholesterol 3 parts.

§ Pellet given at time of transplantation but not found subsequently upon palpation; a second pellet given.

TABLE II: FATE OF SUBCUTANEOUS TRANSPLANTS OF A TESTICULAR INTERSTITIAL CELL TUMOR (2ATE10) IN ESTROGEN-TREATED HYBRID MICE THAT HAD ONE PARENT FROM THE A STRAIN, AND IN MICE OF OTHER STRAINS

| Transplant generation * | Hybrid group ‖ | Group † | Treatment ‡ | No. of mice | Days of treatment | Days treatment was started subsequent to implantation | No. of mice with tumors | No. of mice without tumors |
|---|---|---|---|---|---|---|---|---|
| 4-9 | AC2 | 1 | — | 3 | 129-215 | — | — | 3 |
| | | 2 | SC | 38 | 22-239 | — | 29 | 9 |
| | | 2 | SS | 7 | 149-259 | — | 7 | — |
| | | 3 | SC | 15 | 80-108 | 131-145 | 8 | 7 |
| 5 | AC1 | 2 | SC | 2 | 70-72 | — | 2 | — |
| 5-6 | A72 | 2 | SC | 4 | 105-239 | — | 1 | 3 |
| | | 2 | SS | 9 | 23-322 | — | — | 9 |
| | | 3 | SC | 2 | 108 | 113 | — | 2 |
| 5 | JA2 | 2 | SC | 2 | 74-101 | — | 2 | — |
| | | 3 | SC | 2 | 108 | 131 | 1 | 1 |
| 7-8 | AB1 | 2 | SC | 5 | 68-127 | — | 4 | 1 |
| 7 | CBA | 2 | SC | 3 | 117-196 | — | — | 3 |
| 7 | C3H | 2 | SC | 4 | 49-196 | — | — | 4 |
| 7 | CC1 | 2 | SC | 7 | 39-300 | — | — | 7 |

* See footnotes to Table I.
† " " " " "
‡ " " " " "

‖ First generation hybrids; AC2, C3H ♀ × A ♂; AC1, A ♀ × C3H ♂; A72, C57 ♀ ×A ♂; JA2, JK ♀ × A ♂; AB1, A ♀ × CBA ♂; CC1, C57 ♀ × CBA ♂.

group received subcutaneously either weekly injections of 0.25 mgm. of stilbestrol dissolved in sesame oil or compressed pellets composed of stilbestrol (1 part) and cholesterol (3 parts), the hormone treatment beginning at the same time the tumors were transplanted (Group 2, Tables I and II). The third group received no treatment for different periods subsequent to implantation of the tumors; stilbestrol was then administered either in pellets or oily solution as mentioned above (Group 3, Tables I and II).

Five mice were hypophysectomized after the transplanted tumor had begun to grow. Two were given both estrogen and testosterone propionate subsequent to implantation.

When the animals were killed the skins were reflected to expose the tumors or the sites of implantation. If transplanted tissue was not evident, the areas in which the transplants had been deposited were examined under ultraviolet light. The testes of the estrogen-treated male hosts assumed a bright orange fluorescence when examined in this manner. The persisting fragments of the transplants in animals that did not receive stilbestrol usually fluoresced in the same way, whereas the rapidly growing tissues showed little or no fluorescent material. Areas were removed from most of the tumors for histological examination after staining with hematoxylin and triosin, or with Sudan IV. The fluorescent sites in the subcutaneous areas where the tumors had been implanted but had failed to grow were also removed for histological study.

## OBSERVATIONS

The original tumors used in the experiments to be described are designated 2ATE10 and 3AC1SS. The tumor 2AT10 appeared in a mouse of the A strain that had received weekly injections of 5 mgm. of triphenylethylene for 376 days, beginning at 61 days of age (3). The original tumor consisted predominantly of large interstitial-like cells and blood-filled or fluid-filled cavities, some of which were lined by endothelial cells and others by tumor cells. The tumor 3AC1SS arose in a hybrid mouse ($A♀ \times C3H♂$) that had received 15 mgm. of stilbestrol in sesame oil during 429 days, starting at 42 days of age. The mouse at death had 2 mammary adenocarcinomas and a tumor in the left testis. The tumorous testis measured $11 \times 9$ mm., and was mottled yellow, white, and brown. The left lumbar node was enlarged by metastatic growth. The left testis was composed almost entirely of tumorous tissue; a few atrophic seminiferous tubules alone persisted peripherally. The growth was composed largely of cells with a granular cytoplasm of the type described as of the "second generation" by Hooker and Pfeiffer (10), a few areas of small fibroblast-like cells, and islands of myeloid cells, for the greater part differentiating into polymorphonuclear leukocytes. Only in the latter characteristic did this tumor differ from those extensively described elsewhere (1, 10).

*Gross and histological appearance of the transplanted tumors.*—The transplanted tumors were hyperemic, fleshy, and moderately firm. Sometimes yellowish or tan areas were noted on the surface, especially in those growing in mice of the A strain; these areas gave an orange fluorescence in ultraviolet light. Areas of necrosis were noted sometimes in the center of the tumors, but they usually contained viable and well vascularized tissue throughout. Occasionally 2 or more separate tumors appeared that had apparently arisen from detached foci of cells left along the course of the trochar at the time of transplantation. The growths never became attached to the body wall or invaded the intercostal or abdominal musculature. Occasionally tumors, and even relatively small ones, infiltrated the dermis, denuding the overlying skin and sometimes ulcerating; in such instances the mice generally died of hemorrhage. More usually, however, the tumors were circumscribed and not invasive. Upon incision they bled freely, although hemorrhagic areas were rarely noted. Fragments removed for transplantation crushed readily, and the material was usually of a pulp-like consistency when implanted.

Histologically the transplanted tumors differed considerably. The greater number were composed of cells of rather uniform size, with vesicular nuclei and a granular cytoplasm, supported in a delicate stroma. Sometimes the cells were arranged in cords separated by vascular sinusoids, but usually the growths consisted predominantly of cords or massive groups of cells separated only by capillaries. Some tumors contained areas of smaller, more closely packed, cells with smaller amounts of densely staining cytoplasm, as well as large cells with a cytoplasm that stained lightly with eosin but intensely with Sudan IV. Cells of the former type usually occurred in groups, while those of the latter type were scattered individually about the tumor as well as in groups. A number of tumors, especially those that developed in the hybrid mice, contained scattered areas of tissue showing erythropoiesis or, more rarely, myelopoiesis. Macrophages containing brown pigment, both diffuse and granular, were found in a few of the growths and in the surrounding stroma, especially in those that showed the most intense fluorescence. The intratumoral "brown cells" were noted frequently in those tumors showing scattered necrotic areas.

The transplanted tumors differed from one another, as mentioned above. The spontaneous neoplasms arising in different animals also differed from one another

to a similar but somewhat greater extent (10). The rather wide range of normal variation of the transplanted growths in mice of the same group made it difficult, with the material available, to determine the influence of the several experimental modifications of the environment of the tumors upon any consistent alterations of their histological structure.

*Fate of the transplanted tumors in untreated mice.*—The transplants of one tumor (2ATE10) failed to grow in all 27 mice of the A strain and in the 3 mice of the hybrid groups obtained by mating female mice of the C3H strain with males of the A strain. The 30 mice were observed for 99 to 312 days. Small nodules were palpated at the site of the grafts in 3 of the female mice of this group during the first few weeks subsequent to transplantation, but later they disappeared and were not found at autopsy. It was assumed that they were inflammatory reactions around the implanted tissue, although they might have represented transitory growths that were later resorbed.

When the mice were killed evidences of the grafts were not found in some; in others small pale brownish areas in the subcutaneous tissues were detected, which might have been persisting remnants of the grafts (Figs. 1, 2, 3). These areas were never more than a few millimeters across, had indefinite borders that blended with the surrounding connective tissues, and were not noticeably elevated. The brownish areas were usually so small that they could be detected only when the subcutaneous tissues were inspected carefully. In ultraviolet light they fluoresced a bright orange color, and some could be located only when sought in this manner. When the fluorescent areas were removed and examined histologically they were found to be composed largely of groups of macrophages containing a brownish pigment, some fibroblasts or fibroblast-like cells that might represent transitional interstitial cells, and the usual connective tissue cells. Definite tumor cells could not be identified with certainty, even when serial sections of the sites were examined. The tumor 3AC1SS also failed to grow in 4 hybrid (AC1) hosts that did not receive estrogen (Table III).

*Response of "tumor rests" subsequent to delayed estrogen treatment.*—Estrogens were not administered until 51 to 204 days subsequent to transplantation of the tumor in 17 mice of the A strain (Table I). These mice then were observed for 35 to 222 days, and all but one had growing or large tumors when they were killed. The tumors that developed subsequent to estrogen-treatment were similar in gross and histological structure and in their rate of development to those of the mice that had received estrogen at the time of transplantation.

TABLE III: FATE OF A SUBCUTANEOUSLY TRANSPLANTED TESTICULAR INTERSTITIAL CELL TUMOR (3AC1SS) IN CONTROL AND ESTROGEN-TREATED MICE

| Transplant generation * | Hybrid group ¶ | Group † | Treatment ‡ | No. of mice | Days of treatment | Days treatment started subsequent to transplantation | No. of mice with tumors | No. of mice without tumors |
|---|---|---|---|---|---|---|---|---|
| 1 | AC1 | 2 | SC | 4 | 159-357 | — | 3 | 1 |
| 2 | AC1 | 1 | — | 4 | 91 | — | — | 4 |
| | | 2 | SC | 3 | 89-107 | — | 2 | 1 |
| | | 2 | SS | 6 | 37-111 | — | 6 | — |
| | | 2 | OTP ** | 2 | 330 | — | 2 | — |
| 3 | AC1 | 2 | SC | 3 | 69-75 | — | 3 | — |
| | AC3 | 2 | SC | 3 | 50-91 | — | 3 | — |
| 4 | A | 2 | SC | 3 | 272-289 | — | — | 3 |
| | C3H | 2 | SS | 6 | 244-394 | — | — | 6 |
| | AC1 | 2 | SC | 3 | 99-288 | — | 2 | 1 |
| | AC1 | 3 | SC | 1 | 39 | 162 | 1 | — |

* See footnotes to Table I.
† " " " " "
‡ " " " " "
¶ First generation hybrid mice; AC1, A ♀ × C3H ♂; AC3, C3H ♀ (without milk influence) × A ♂.
** OTP—16.6 mgm. estradiol benzoate and 1 mgm. testosterone propionate weekly.

In order to compare the rate of development of the "tumor rests" with recently transplanted fragments of tumor the following experiments were undertaken. Five young male and female mice of the A strain received grafts of tumor 2ATE10 in the subcutaneous tissues on the right side. Ninety-nine days later a second transplant was made on the left side, and a pellet of stilbestrol-cholesterol was implanted on the right side. When these mice were killed 76 to 167 days later tumors were present on both sides; the tumors on the two sides showed no consistent differences in size (Figs. 4, 5). The "rest" developed as rapidly as did the recently transplanted tissue in 2 mice and more slowly in 3.

Nineteen mice of the hybrid groups received transplants 80 to 108 days before estrogen was administered

and no tumors developed (Group 3, Table II). Subsequent to estrogen treatment the tumors grew at the sites of transplantation in 9 mice. Although the incidence of growth of the transplants was lower among this group than among the mice of the A strain (Group 3, Table I), the incidence was also lower among the hybrid mice that received estrogen and tumor grafts simultaneously.

One hybrid mouse that received the tumor 3AC1SS 162 days prior to receiving estrogen had tumors measuring $10 \times 5$ and $5 \times 5$ mm. when killed 39 days later (Table III).

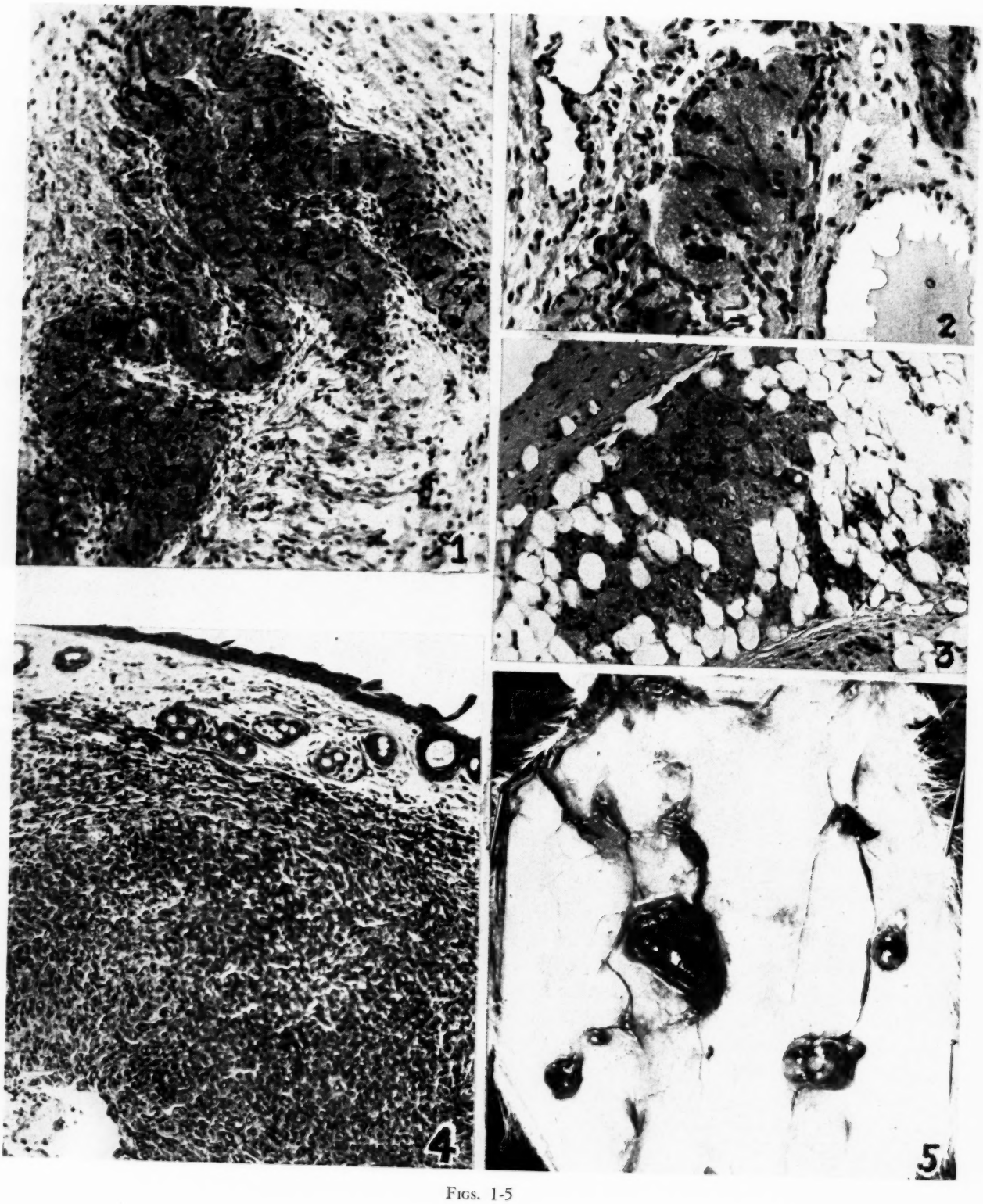
*The effect of discontinuing the injection of estrogen on the transplanted tumors.*—Ten mice of the A strain, 9 of which grew transplants of tumor 2ATE10, received weekly subcutaneous injections of 0.25 mgm. of stilbestrol for 82 to 142 days, beginning at the time the tumors were transplanted. Subsequently estrogen was not given for 14 to 188 days, during which time the growth of the tumors was observed. When the treatment was discontinued the tumors all exceeded 10 mm. in their greatest dimension. The development of the tumors following the discontinuance of estrogen treatment was comparable to that of the controls. The irregular rate of development of the tumors in the different animals of the two groups made a quantitative evaluation of tumor growth difficult. The observations demonstrated, however, that growth continued or that the tumor persisted subsequent to the discontinuance of treatment. Histologically the tumors of the mice that had not received estrogen for 2 to 6 months exhibited a range of structure similar to those of the continuously estrogen-treated mice, with only an indication that: (a) an increased number of them showed more numerous areas of local necrosis and more macrophages containing brown pigment; and (b) a greater number contained smaller cells with more deeply staining cytoplasm and smaller nuclei.

*Growth of transplants in mice receiving both estrogens and androgen.*—Two mice of the AC1 hybrid group bearing transplants of the 3AC1SS tumor received weekly subcutaneous injections of 16.6 $\mu$gm. of estradiol benzoate and 1 mgm. of testosterone propionate in 0.05 cc. of sesame oil. Tumors were not detected in either until approximately 8 months subsequent to transplantation, or 3 to 4 months after the 6 estrogen-treated controls had died or been killed, all bearing large tumors. Tumors subsequently developed, and large ones were present when the hosts were killed 11 months subsequent to receiving the transplant (Table III). Although the number of mice was small the testosterone appeared to inhibit the rate of development of the tumors, an interesting observation because the tumors themselves produce some androgen (9, 10).

*Growth of transplanted testicular tumors in hypophysectomized mice.*—The tumors were transplanted into 6 estrogen-treated mice, which were hypophysectomized 41 or 83 days later. One mouse died during the operation and is not included in Table IV. The mice survived hypophysectomy for 10 to 28 days and lost 3 to 6 grams in weight during this time. All still carried pellets of stilbestrol. During the period subsequent to hypophysectomy the tumors showed no consistent changes in size. Likewise during a period of 28 days the tumors of 2 intact mice did not show significant measurable changes in size. A tumor failed to develop in one control mouse. Morphologically the growths of the hypophysectomized mice were comparable, with one exception, to those of the controls. The one mouse that survived 28 days had a tumor consisting of cells resembling "generation two," as described by Hooker and Pfeiffer (10), but their cytoplasm was somewhat reduced in amount and the nuclei were smaller and stained more intensely. Part of the growth contained cells that were predominantly elongated and spindle-shaped.

*The rate of growth of transplanted tumors in successive transplant generations.*—The transplanted tumors grew slowly. Usually they first become detectable by palpation within 20 to 30 days, although in some mice they were first noted from 50 to 60 days subsequent to transplantation. They grew slowly during the following 3 to 4 months, and from that time on changed comparatively little as a rule in size. The first transplantation of the grafted tumor (2ATE10) was performed 115 days after the host had received the tumor. Subsequent transplantations were made from mice that had carried the transplants for 43 to 175 days. Most of the tumors attained diameters of 1 to 3 cm. within 70 to 90 days. After the first transfer the tumors grew at about the same rate during the several succeeding ones. The transplanted tumors obtained from the original neoplasm grew more slowly than those obtained later. Some mice lived 245 days with the tumors (Tables I to III). The structure of the tumor 2ATE10 likewise retained a constant norm during a 3 year period.

*Growth of the tumors in mice of unrelated strains or in hybrid mice.*—The tumor 2ATE10 failed to grow in mice of two inbred strains other than the A strain; namely, the C3H and CBA strains. Hybrid mice obtained by crossing members of the A strain with those of the C3H, CBA, C57, or JK strains grew the transplanted tumors, although the incidence of successful transfers was lower than in mice of the pure strains (Table II). Hybrid mice with one or the other parent from the A strain were themselves




FIGS. 1-5

susceptible to the acquisition of testicular tumors subsequent to estrogen treatment.

The tumor that arose in the mouse of the AC1 group (3AC1SS) failed to grow in mice of either of the parental stocks, but grew in those of the hybrid groups AC1 and AC3 (Table III). In this respect the transplanted testicular tumors resembled other transplanted tumors of mice that have been studied. Even an environment high in estrogenic hormone did not overcome the genetic incompatibility of host and transplant. regulated the transplantability of mammary adenocarcinomas in mice (18). Some tumors arising in mice of pure strains would grow in all first generation hybrids obtained by mating animals of the tumor strain with other strains, but in only a few of the $F_2$ generation.

Although no attempt was made to study the genetic factors involved in the transplantation of testicular tumors, the observations that were made indicate that these are much the same as for the mammary ade-

TABLE IV: INFLUENCE OF HYPOPHYSECTOMY ON GROWTH OF TRANSPLANTED TESTICULAR TUMORS IN ESTROGEN-TREATED MICE OF THE A STRAIN

| Transplant generation and number of animal | Age of transplant at hypophysectomy, days | Size of tumor at operation, mm. | Weight at hypophysectomy, gm. | Postoperative period survival, days | Size of tumor at death, mm. | Weight of animal at death, gm. |
|---|---|---|---|---|---|---|
| T3 7 | 41 | 7 × 13 | 19.8 | 10 | 8 × 13 | 14.2 |
| T3 8 | 41 | 7 × 13 | 17.8 | 15 | 9 × 17 | 14.7 |
| T4 7 | 83 | 13 × 19 | 19.6 | 28 | 13 × 14 | 16.1 |
| T4 8 | 83 | 11 × 21 | 18.3 | 15 | 12 × 20 | 12.4 |
| T4 9 | 83 | 7 × 11 | 20.5 | 11 | 7 × 10 | 15.5 |
| T4 10 | Control | 12 × 19 | 20.5 | 28 | 11 × 22 | 21.7 |
| T4 11 | Control | — | 20.7 | 28 | — | 22.4 |
| T4 12 | Control | 11 × 18 | 22.5 | 28 | 10 × 19 | 26.1 |

TABLE V: COMPARISON OF GROWTH RATE OF "TUMOR RESTS" AND OF GRAFTS INOCULATED COINCIDENTLY WITH IMPLANTATION OF ESTROGEN PELLETS

| Transplant generation and number of animal | Period observed after graft on right side and before estrogen treatment, days | Period observed after estrogen and after transplanting tumor on left side, days | Size of tumor on right side, mm. | Size of tumor on left side, mm. |
|---|---|---|---|---|
| T3 7 | 99 | 76 | 10 × 13 | 6 × 10 |
| T3 8 | 99 | 101 | 4 × 7 | 12 × 23 |
| T3 9 | 99 | 167 | 10 × 14 | 15 × 19 |
| T3 10 | 99 | 161 | (See Fig. 5) | (See Fig. 5) |
| | | | 15 × 15 | 28 × 15 |
| T3 15 | 99 | 76 | 6 × 15 | 3 × 3 |
| T3 16 | 99 | 76 | 8 × 20 | 7 × 14 |

## DISCUSSION

The testicular interstitial cell tumors that arose in estrogen-treated mice have grown progressively only in other estrogen-treated animals that were genetically related to the donor. Several years ago it was noted that the genetic constitution of the hosts and tumors nocarcinomas. The tumor that arose in a mouse of a pure strain would grow in other mice of that strain and in $F_1$ hybrids obtained by crossing the strain of origin to foreign strains, but not in mice of foreign strains; the tumor that arose in a hybrid mouse would not grow in either parental strain.

## DESCRIPTION OF FIGURES 1 TO 5

FIG. 1.—Section through a remnant of tumor 2ATE10 persisting in a hybrid mouse 158 days after transplantation. Although this mouse had received estrogen the transplant failed to grow. The remnant consisted predominantly of large, brown, multinucleated cells. Mag. × 100.

FIG. 2.—Same as Fig. 1. Mag. × 200.

FIG. 3.—Section of transplant of tumor 2ATE10 that persisted 366 days in a mouse of the A strain that did not receive estrogen. The remnant showed an orange fluorescence when examined under ultraviolet light. Mag. × 100.

FIG. 4.—Section of transplanted tumor (2ATE10) that grew in the subcutaneous tissue of an estrogen-treated mouse of the A strain for 73 days.

FIG. 5.—Skin and tumors of mouse T3-10 (See Table V). Tumors on right side were transplanted 99 days before those on left. Pellet of stilbestrol-cholesterol seen just anterior to smaller tumor on right side was implanted same time as tumor on left side.

Unlike the mammary tumors referred to above the testicular tumors required an environment that contained estrogen in amounts greater or more persistent than that occurring in normal females. In the absence of an adequate hormonal environment the grafts regressed when transplanted into the subcutaneous connective tissues, but small amounts of viable tumor persisted for periods up to 7 months. Many of these persisting tumors were so small that they could not be detected, or were detected only as small pigmented areas. Histologically the cells in these "rests," even when they could be identified, did not resemble those found in most of the growing tumors, yet cells that were capable of proliferation persisted and grew when the animals were treated with estrogen. The "rests" failed to grow during periods of observation up to 312 days when the hosts received no estrogens.

The persistence of the transplants in a dormant state, yet in a state in which they were capable of growth if given a proper environment, is interesting in connection with the "embryonic rest" theory of cancer. The transplants were no more than potential sources of tumors for periods up to 7 months, unless a suitable environment was made available for their proliferation by adding estrogen. Because few testicular interstitial cell tumors have arisen in mice that have not received estrogen it might be assumed that the environment also is important in their etiology. The fact that a few interstitial cell tumors do arise in mice that have not received estrogen might indicate, however, that all interstitial cell tumors do not require an environment high in estrogen for their growth (4). One testicular tumor, which arose in an estrogen-treated mouse and grew in untreated mice subsequent to transplantation, has been described by Bonser (2). In the untreated it grew most consistently in females, but grew equally well in both estrogen-treated males and females. These two facts afford interesting points for speculation. For example, the testes of many mice of strains susceptible to testicular tumors subsequent to estrogen-treatment, may contain or acquire tumorous foci that are dormant for want of a proper environment for development. The estrogen, which has been assumed to be the carcinogenic stimulus for testicular tumors, either directly, or indirectly through the hypophysis, may merely stimulate growth of these neoplastic foci, the foci being more subtly determined by genetic factors. The strain-limited appearance of such tumors might argue further for this hypothesis. It must not be overlooked, however, that the hereditary mechanism may affect some specific humoral influence that will induce the formation of tumorous foci. Another explanation for the strain-limited development of testicular tumors involves possible differences in the gonadotropic stimulating activity of the pituitary (16).

Although the original tumors had metastasized to the lumbar or renal lymph nodes, metastatic growths were never detected in mice bearing the transplanted tumors. Occasionally the regional lymph nodes were invaded when in contact with the tumors, although it was not uncommon to find a normal lymph node partially surrounded by tumor. The skin overlying the grafts was not infrequently invaded, with loss of the overlying hair, ulceration, and death eventually from hemorrhage or infection. The transplanted tumors cannot be considered particularly malignant.

The capacity of the tumors to persist and even to grow after the cessation of estrogen treatment indicates that once they have become established their autonomy increases; that is, they are not reversible, even when estrogen is withheld.

The presence of hematopoietic tissue was noted in many of the grafts, although not in the sections that were examined from all of them; the greatest amount occurred in transplants of the 2ATE10 tumor that grew in hybrid mice. Hematopoietic activity has been described in testicular tumors arising in estrogen-treated mice of the JK (3) and A (2) strains. It is possible that some undifferentiated or relatively omnipotent cells having the capacity to differentiate into normoblastic or myeloblastic cells, as well as into interstitial cells, were carried over with the tumors. Extramedullary myelopoiesis is not uncommon in the adrenals or spleens of mice, but it has not been observed in non-tumorous testes. That these growths are not teratomatous was indicated by the failure to detect epithelial tissues, or mesodermal tissues other than those mentioned above.

That few viable tumor cells characteristic of the tumors growing in estrogen-treated mice persisted in the untreated mice was shown by direct histological observation. Most of the transplants in untreated mice consisted largely of macrophages containing a brown pigment, and designated "brown cells." The close correlation between the presence of pigment and the capacity to fluoresce in ultraviolet light indicates that the pigment possesses this capacity rather than the contents of the tumor cells. The growing transplants contained very little or no material giving a bright orange fluorescence under ultraviolet light. The testes of estrogen-treated mice, as well as the "rests" that could be identified following the transplantation of tumors into untreated mice, both contained "brown cells," and both exhibited a brilliant fluorescence.

## SUMMARY

1. Two testicular interstitial cell tumors, one that arose in a mouse of the A strain that had received

triphenylethylene and one that arose in a hybrid mouse (A♀ × C3H♂) that had received stilbestrol were transplanted subcutaneously into other mice for 9 and 4 generations respectively. The tumors consisted predominantly of cells of "generation two," as described previously.

2. The tumors failed to grow in all 34 genetically susceptible and untreated mice into which they were implanted.

3. Seventeen mice of the A strain received transplants and no growth occurred during the subsequent 51 to 204 days. Estrogens given at this time resulted in the growth of 16 of the "tumor rests." The rests grew as rapidly as the recently transplanted tumors.

4. Transplanted tumors that developed in estrogen-treated mice continued to grow, or persisted, when treatment was discontinued for periods of 14 to 188 days.

5. The tumors persisted for 10 to 28 days in hypophysectomized mice although the hosts lost weight.

6. The rate of growth and the structure of the tumors did not change appreciably during a period of 3 years of successive transplantations.

7. The testicular tumors failed to grow in estrogen-treated mice of unrelated strains.

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# The Incidence of Adrenal Cortical Carcinoma in Male Mice of the Extreme Dilution Strain over One Year of Age*

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The effect of gonadectomy on the incidence of adrenal cortical carcinoma has been reported for male mice of the ce strain up to 1 year of age (5). Observations on ce strain intact and castrated male mice over 1 year of age are presented in this report.

## MATERIALS AND METHODS

The material was obtained from JAX ce strain (2) male mice. Sixteen intact males and 23 males castrated when from 1 to 3 days old were autopsied at monthly intervals when they were from 13 to 29 months of age. The age at autopsy is given in Tables I and II.

The method of study has been described (2, 3, 5).

Table I: Observations on Intact Male Mice of the ce Strain over 1 Year of Age

| Age, months | Number examined | Sub-maxillary gland | Accessory sex organs | Adrenal cortical tumors | Mammary glands | Average body weight, gm. |
|---|---|---|---|---|---|---|
| 13 | 1 | ♂-type | normal | none | rudimentary | 30 |
| 15 | 1 | " | " | " | " | — |
| 16 | 3 | " | " | " | " | 33 |
| 18 | 2 | " | " | " | " | 29 |
| 19 | 2 | " | " | " | " | 30 |
| 21 | 2 | " | " | " | " | 29 |
| 23 | 2 | " | " | " | " | — |
| 24 | 1 | " | " | " | " | 28.5 |
| 25 | 2 | " | " | " | " | 31.5 |
| 29 | 1 | " | " | " | " | — |

## RESULTS

*Frequency of adrenal cortical tumors.*—No carcinomas of the adrenal cortex were found in the 16 intact male mice (Table I), whereas at least one tumor was discovered at this site in each of the castrated males; in 20 of the 23 in the left, and in 17 in the right adrenal. Fourteen had bilateral growths and 9 had tumors in one adrenal only. Except in the 2 mice that had small tumors in each adrenal (P1700 and P2016), the tumors were identified as to side (Table II). All were classified as to size. Of the 21 mice where size and side data were both obtained, there were 15 with the larger tumor on the left side and 6 with the larger tumor on the right. Both males and females (4) of this strain have thus had tumors with greater frequency, and more often of larger size, on the left than on the right side.

*Size of adrenal tumors.*—Castrated males had adrenal neoplasms of smaller size at given comparable ages before 1 year than ovariectomized females (5); beyond 1 year of age the tumors also averaged smaller in these males than in the ovariectomized group (4). The size for the castrated mice is shown in Table III, the number and percentage in each of 3 size groups for males and females in Table IV.

*Histology of adrenal tumors.*—The histology of all adrenal growths in this series has been described (1) except for those in 2 animals; a brief description of these is given below.

P2790, castrated male, autopsied when 27 months of age, had two adrenal tumors. On the left side the growth measured cephalocaudally 21 mm., median-laterally 19 mm., and dorsoventrally 15 mm. Its major part consisted of typical, diffusely arranged, large polygonal, adrenal cortical tumor cells. Where

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these cells and this cellular arrangement predominated the tumor tissue is called "Type I"; see (1) for more detailed type description. Part of the growth was made up of small cuboidal cells with dark-staining nuclei. These cells showed a tendency to form rows and cords, and to surround areas composed of giant cells with yellow pigmented cytoplasm. Tumor tissue of this type is here called "Type II." A few normal cortical cells and small areas of brown degeneration were present at one place on the periphery of the neoplasm. As previously noted (2) serial sections of all of each tumor, when large, were not made.

TABLE II: ADRENAL TUMOR SIZE, AND SIDE IN RELATION TO SIZE, FOR 23 CASTRATED MICE OF THE CE STRAIN OVER 1 YEAR OF AGE

| Animal number | Age, months | Adrenal tumor, left side | Adrenal tumor, right side | Left side, larger | Right side, larger |
|---|---|---|---|---|---|
| P2744 | 13 | + | 0 | + | — |
| P2433 | 14 | 0 | + | — | + |
| P2478 | 15 | 0 | + | — | + |
| P2320 | 15 | + | 0 | + | — |
| P2415 | 17 | + | 0 | + | — |
| P2718 | 17 | + | 0 | + | — |
| P2684 | 17 | + | + | — | + |
| P2683 | 18 | + | + | + | — |
| P1700 * | 18 | + | + | — | — |
| P2525 | 19 | 0 | + | — | + |
| P2528 | 21 | + | + | + | — |
| P2578 | 21 | + | + | + | — |
| P2577 | 21 | + | 0 | + | — |
| P2530 | 23 | + | + | + | — |
| P2543 | 23 | + | + | + | — |
| P2529 | 23 | + | + | + | — |
| P2537 | 23 | + | + | — | + |
| P2283 | 24 | + | 0 | + | — |
| P2016 * | 24 | + | + | — | — |
| P2576 | 25 | + | + | + | — |
| P2791 | 27 | + | + | + | — |
| P2790 | 27 | + | + | + | — |
| P2640 | 28 | + | + | — | + |
| | | — | — | — | — |
| Total (23) | | 20 | 17 | 15 | 6 |

* Side not known.

In place of the right adrenal there was a growth measuring cephalocaudally 16 mm., median-laterally 19 mm., and dorsoventrally 14 mm., which was similar in structure to that on the left side; both cortical and medullary cells were found at its periphery.

P2791, a full brother to P2790, was autopsied when 27 months of age. Two adrenal tumors were present. In place of the left adrenal there was a growth that measured cephalocaudally 14 mm., median-laterally 9 mm., and dorsoventrally 9 mm. In place of the right adrenal there was a tumor that was nearly round and measured 4 mm. in diameter. Both were similar in structure to those in P2790, but with a higher proportion of Type II tumor tissue. The right neoplasm was surrounded, in the sections observed, by a thin layer of cortex, which in places had compact areas of Type A cells (2). Medullary cells were grouped beneath the cortex in one region, and small areas of brown degeneration were present within the median edge of the cortex.

*Accessory sex organs.*—No abnormalities of the seminal vesicles and prostate were observed in the intact mice; their size was interpreted as normal.

On the other hand, various types and degrees of development were found in these accessory sex organs in the castrated males. In 12 of the 23 castrates they were small (Table III). In one of these, P2640, 28 months of age, the seminal vesicles were enlarged slightly, but had low columnar epithelium and very little secretion.

The seminal vesicles and prostate were grossly enlarged in the remaining 11 castrates (Table III). They were especially large in P2320 and P2718 (judged to be over ½ normal size), but in no case were they as large and as turgid as in intact ce strain males of similar age. The secretion was copious. Histologically the glands were similar to those of intact males.

Squamous metaplasia of the epithelial lining of the prostate, a condition that has been observed after treatment with estrogens, was found in 4 mice; P2433, P2415, P2578, and P2577; (for an illustration of squamous metaplasia in a castrated ce strain mouse see [5] Fig. 15). In all these mice the metaplasia was 4 to 5 cells thick, without a keratinized layer. Material within the prostatic tubules varied between individuals. P2433 had keratinized material with very few leukocytes; P2415 was similar, except that there were many leukocytes. Nests of leukocytes were also present along the outer edge of the epithelial layer, as may occur in the vaginal epithelium at postestrus. P2578 had nucleated epithelial cells and a few leukocytes in the lumen. P2577 had material in the lumen interpreted as keratinized material that had undergone considerable degeneration. In this mouse part of the prostate had columnar epithelium and evidence of apparently normal secretion. Seminal vesicle secretion was copious.

In all these latter 4 mice there was an excess of connective tissue stroma around the tubules. P2415 had hyalinized connective tissue (as in [5] Fig. 13).

*Mammary glands.*—The skins of all the intact males were examined in order to observe the mammary glands; when present these were rudimentary (Table I).

The mammary glands of 21 castrated males were examined (Table III). In 10 no duct development was found; the amount varied greatly in the remaining 11, not only from individual to individual but from gland to gland within individual mice. One

reason for variation within individuals was the fact that some of the glands failed to appear, perhaps because there had been no glandular rudiment in which growth could occur. All 10 glands had developed in only 1 mouse, P2718, autopsied at 17 months. In general, the ducts in these 11 mice were only moderately developed; that is, they were medium long, medium branched with few small lateral branches, and narrow throughout.

End buds, indicating rapid duct growth, were not found. Alveoli were present in 4 mice; P2415, P2718, P2528, and P2578. In only one, P2528, was alveolar development extensive and advanced; secretory droplets were present both within the cells and in the lumen (Fig. 1).

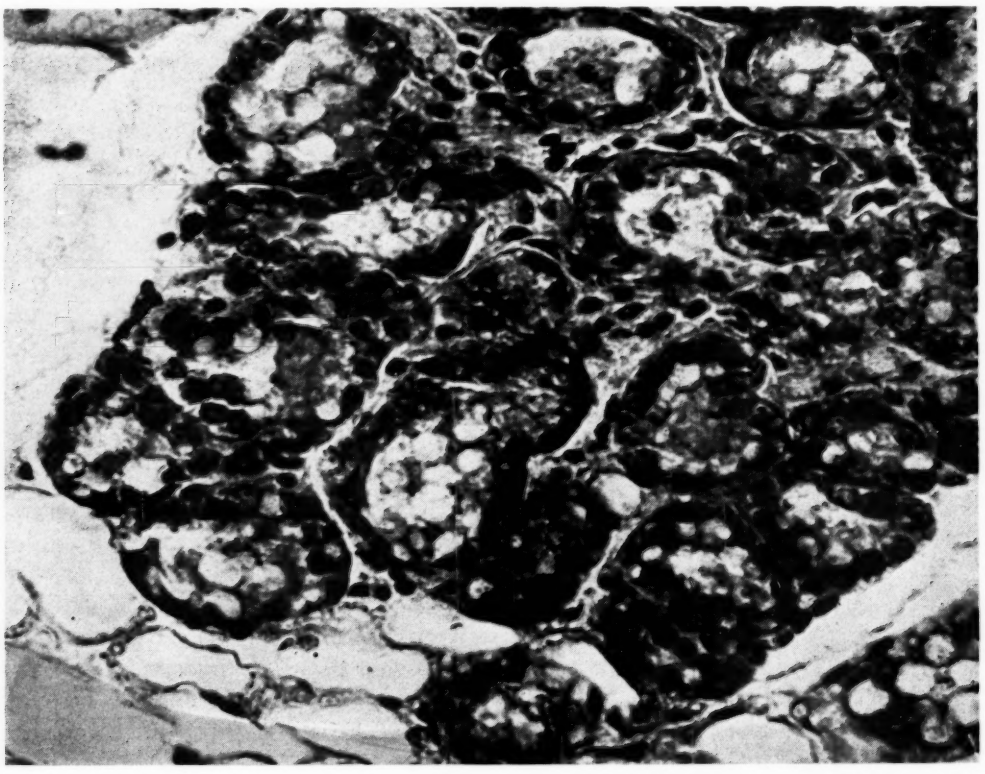

FIG. 1.—Section of a mammary gland. Note extensive alveolar development. Gonadectomized male, P2528, 21 months of age. Mag. × 490.

*Submaxillary glands.*—The submaxillary glands of all intact males were of the normal male type.

Those of the castrated males varied from individual to individual. It was found, however, that they could be classified in 3 groups; male type, intermediate (male-female) type, and female type. On this basis there were 10 male type, 9 intermediate,[1] and 4 female type. The mice are identified in Table III.

*Pituitary glands.*—Some abnormalities of the pituitary gland were observed in the gross. In P2528 it was greatly enlarged, particularly the right side of the anterior lobe. Localized hyaline areas were present in P2791 and P2790, in the posterior edge of the anterior lobe and unilaterally in both pituitaries. The pituitaries were not differentially stained for a study of cell types.

[1] This includes glands from some of the mice that were in poor health.

## DISCUSSION

From the results obtained it is clear that castration at an early age in ce strain mice led to the appearance of adrenal cortical carcinomas. Also it is true that these tumors eventually appeared in a high percentage of the castrated mice (in 100 per cent of those in the present experiment). It is also interesting to think of the experimental results in another way. That is, in some way the testes completely protected ce strain mice from the incidence of adrenal cortical carcinomas (100 per cent protection in the present experiment). The method of protection seems to be generalized, in so far as this may come from either ovaries in females (2, 4) or testes in males. Experimentation with specific endocrine preparations should be of value in further explaining the mechanism responsible. It has been suggested that the action may be through the pituitary (2).

Although there is evidence of similarity in hormone secretion between ce strain castrated males and ovariectomized females as the adrenal tumors develop,

TABLE III: OBSERVATIONS ON CASTRATED MALE MICE OF THE CE STRAIN OVER 1 YEAR OF AGE

| Animal number | Size of tumor | Sub-maxillary gland | Accessory sex organs, size | Mammary glands | | | Body weight, gm. |
|---|---|---|---|---|---|---|---|
| | | | | Duct growth | End buds | Alveoli | |
| P2744 | small | intermediate | small | 0 | 0 | 0 | 32 |
| P2433† | medium | ♀ type | " * | + | 0 | 0 | – |
| P2478† | small | " " | " | – | – | – | – |
| P2320 | medium | ♂ type | medium | + | 0 | 0 | – |
| P2415 | " | intermediate | " * | + | 0 | + | – |
| P2718 | large | ♂ type | large | + | 0 | + | – |
| P2684 | medium | " " | medium | 0 | 0 | 0 | 30 |
| P2683† | small | intermediate | small | 0 | 0 | 0 | 30 |
| P1700 | " | " | " | – | – | – | – |
| P2525 | " | ♀ type | " | 0 | 0 | 0 | – |
| P2528 | medium | ♂ " | medium | + | 0 | + | – |
| P2578 | " | ♀ " | " * | + | 0 | 0 | – |
| P2577 | large | ♂ " | " * | + | 0 | + | 31 |
| P2530† | medium | intermediate | small | 0 | 0 | 0 | – |
| P2543† | small | " | " | 0 | 0 | 0 | – |
| P2529 | large | ♂ type | medium | + | 0 | 0 | – |
| P2537† | small | intermediate | small | 0 | 0 | 0 | – |
| P2283† | " | " | " | 0 | 0 | 0 | – |
| P2016 | medium | ♂ type | " | + | 0 | 0 | – |
| P2576† | small | intermediate | " | 0 | 0 | 0 | – |
| P2791 | medium | ♂ type | medium | + | 0 | 0 | 30 |
| P2790 | large | " " | " | 0 | 0 | 0 | 35 |
| P2640 | " | " " | " | + | 0 | 0 | 27 |

*Squamous metaplasia of prostatic epithelium.
†Animal in poor health at time of autopsy.

TABLE IV: SIZE OF ADRENAL TUMORS * IN MICE OF THE CE STRAIN OVER 1 YEAR OF AGE

| | Total number | Large | | Medium | | Small | |
|---|---|---|---|---|---|---|---|
| | | Number | Per cent | Number | Per cent | Number | Per cent |
| Castrated | 23 | 5 | 22 | 9 | 39 | 9 | 39 |
| Ovariectomized | 41 | 33 | 81 | 5 | 12 | 3 | 7 |

* Estimated size of tumor.
Under 0.5 cm. in diameter = small.
0.5 cm. to 1.5 cm. in diameter = medium.
Over 1.5 cm. in diameter = large.

it is not clear what real differences there may be, if any. The difficulties involved in interpretation are numerous; for example: (a) it is not known how long a tumor may have been present in any one mouse; (b) there is a regular difference in tumor size in males and females; and (c) it has been necessary to interpret the data on different organs in the two classes of animals. Transplantation studies are in progress, which may eliminate some of these difficulties.

## SUMMARY

Two groups of strain ce mice were studied: (a) 23 castrated male mice, and (b) 16 intact male mice. Castration was performed when the mice were 1 to 3 days of age. The animals were autopsied at advanced ages, beginning when the first were 13 months of age. Adrenal cortical carcinomas were found in 100 per cent of the castrated mice and in none of the intact.

The condition of the accessory sex organs of the castrated mice indicated that they were being subjected to influences similar to the sex hormones, that these were multiple, and that their amount and nature varied from individual to individual.

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# Estrogen Production by Sertoli Cell Tumors of the Testis*

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There is a group of tumors of the testis in dog and man that cause feminization as a result of estrogen production. In this paper we shall demonstrate that these are neoplasms of the sustentacular cells of Sertoli which, in the dog at least, contain large amounts of estrogen.

*Feminizing testicular tumors.*—The syndrome of testicular tumors in dogs producing estrogenic effects was described by Greulich and Burford (13). The dogs are sexually attractive to other males (13, 33, 34), the tumors are often in cryptorchid testes (13, 19), the mammary glands are enlarged (13, 33), and generalized alopecia with increased pigmentation of the abdominal skin and swelling of the penile sheath are sometimes present. Squamous metaplasia of the epithelium of the prostate and posterior urethra is usual (11, 34).

The tumors often present similar cytologic characteristics. In the cases of Greulich and Burford they consisted of tubular structures of microscopic size. Innes (19) classified the growths as tubular adenomas (Sertoli cell tumors), and stated that some were of a strictly tubular variety while in others the tumor cells had transgressed the confines of the tubule to form irregular lobular masses. Zuckerman and McKeown (34) classified these neoplasms as adenocarcinoma, and considered that "essentially they represent a malignant development of all the cells of the seminiferous tubules, including the cells of Sertoli." The occurrence of tubular adenomas has been recognized, apart from the association with feminized males, for some time; thus Peyron (25) gave a histologic description of Sertoliform tumors of the testis in dogs more than 20 years ago.

Pick (26) described yellow nodular formations of cylindrical neoplastic cells arranged in tubules in the ovary and in the cryptorchid testis of man, which he called *adenoma tubulare ovarii testiculare;* the cells were about 15$\mu$ in height and consisted of clear cytoplasm with basal nuclei. Confirmatory studies of the pathology of these nodules by several workers have been reviewed by Krückmann (20).

Neoplasms of the human testis associated with growth of the breast have been reported frequently; commonest among them is the chorionic epithelioma (reviewed by Gilbert, 12). Three examples of testicular tumors with accompanying mammary hyperplasia have been interpreted as interstitial cell growths; the tumor cells were not arranged in tubular form. The case of Monaschkin (23) concerns a tumor of round, vacuolated neoplastic cells, which were stated to resemble the interstitial cells of Leydig. Hunt and Budd (4, 18) reported a man with symptoms of impotence and enlarged painful breasts, which subsided after removal of the associated testicular tumor. The growth was yellow and lobulated; the cells were ovoid in shape with eccentrically placed nuclei containing one or more large, basophilic nucleoli. Nation, Edmondson, and Hammack (24) described a man with mastopathy that did not disappear following removal of a testicular tumor. The cells of this neoplasm contained oval nuclei with one or more large, eccentric nucleoli; many fine droplets of fat were visible in the cytoplasm.

*Estrogen production by the testis.*—It is well known that the testis produces estrogen. Thus Fellner (11) observed that lipoid extracts of bull testis injected into castrate guinea pigs caused growth of the uterus. Qualitative studies indicating the presence of estrogen in this gland have been reported by others, also, for the bull (3, 8, 9, 21), and by still others for the deer (7). Zondek (32) found that the urine of stallions contained, on the average, 170,000 mouse units of estrogen per liter, while the urine of colts and geldings had about 0.3 per cent of this amount. Furthermore, the testis of the stallion contained 23,100 mouse units of estrogen in 350 grams, but less than 0.09 per cent of this amount was extracted from bull testis. From horse testes Beall (1) isolated $\alpha$-estradiol (0.21 mgm. per kgm.) as its di-$\alpha$-naphthoate, and estrone (0.36 mgm. per kgm.) as its 3,5-dinitrobenzoate; these are higher than the estrogenic values reported for any other tissue.

* This investigation was aided by grants from the Committee for Research in Endocrinology, The National Research Council; and from the Albert and Mary Lasker Foundation, Inc. and the Sidney and Frances Brody Foundation.

## METHODS

Five dogs with feminizing testicular tumors of spontaneous origin were available for study. Unless lactation was present, prolactin, 15 mgm. daily for 3 days, was injected subcutaneously. At autopsy the pituitary, thyroids, adrenals, testes, and prostate were weighed, and histologic preparations made of these tissues and of the breast. The tissues, and also normal testis from man and the dog, were stained for fat with Sudan, using frozen sections and the gelatin [1] technic of Heringa and tenBerge (14). Water and total fat content of the testes were determined as previously described (16).

Formalin-fixed testicular tumors of 2 dogs and, as a control, follicle-containing ovaries of 5 dogs were extracted for estrogen with fat solvents as follows: About 13 to 70 gm. of the tissues were minced, weighed, and extracted in a Soxhlet flask for 24 hours respectively with 95 per cent ethanol, ethyl ether, and again 95 per cent ethanol. The fat solvents were combined and evaporated to dryness. The residue was dissolved in ethyl ether, washed 4 times with distilled water, and evaporated to dryness. The residue was again taken up in 95 per cent ethanol, diluted with water to reduce the concentration of alcohol to 70 per cent, and extracted twice with petroleum ether in a separatory funnel; petroleum ether was discarded and the ethanol fraction evaporated to dryness. The residue was dissolved in sesame oil.

Bioassay was done on immature female albino mice, 21 days of age and weighing 6 to 8 gm., by the method of Evans, Varney, and Koch (10). Five mice were used for each assay level, and 10 for the controls.

## RESULTS

*Anatomic status.*—The animals were stray dogs of mongrel type and weighed between 9.3 and 13.6 kgm. The ages were unknown, but 4 of the animals were old since they had dense cataracts and worn incisors; 1 dog with bilaterally cryptorchid testes was considerably younger, for it had normal eyes and teeth.

The dogs were sexually attractive to other males. All had hypertrophy of the mammary papillae (Fig. 1); 1 dog lactated spontaneously, 2 lactated after prolactin injections, and in 2 dogs lactation was not induced. In the dogs that lactated 0.5 to 1.0 cc. of lipid-rich milk was expressed from each of the papillae.

The prostate gland weighed from 6 to 38 gm. Squamous metaplasia of the prostatic epithelium was present in all cases. In the smallest gland the acini were atrophic, and the keratinized epithelium characteristic of estrogen dominance was present only in the prostatic urethra; in the other glands metaplasia was present throughout the acini also.

Constant changes were not observed in the suprarenal glands; the weight of a single gland varied from 0.334 to 2.0 gm. In 2 dogs the suprarenals were small and there was atrophy of the cortex with "brown degeneration," a lipoid band in the cortex encircling the medulla as described by Cramer and Horning (5, 6) in certain strains of mice. In 2 dogs the suprarenals were normal and in 1, whose suprarenals weighed 1.48 and 2.0 gm., small cortical adenomas were present. The pituitaries weighed from 35 to 73.5 mgm.; neither they nor the thyroids were remarkable. One of the dogs had a squamous carcinoma of the anus with retroperitoneal metastasis in lymph nodes.

The testicular tumors were 7 in number, unilateral in 3 dogs and bilateral in 2; 3 of the growths were in cryptorchid testes, bilateral in 1 animal. Metastases were not present. The nontumorous testicular tissue showed moderate to severe atrophy of the germinal epithelium in all the dogs. The feminizing neoplasms of the dog testis correspond to the lobulated tumors with high fat content of Huggins and Eichelberger (16); in the gross they are firm, white in color, and are bossed in contradistinction to the soft orange-yellow interstitial cell tumors, which usually occur as single discrete masses.

In 3 of the growths the cells were arranged in tubules; in 2 the cells were diffuse (Fig. 2) and tubular arrangement was not apparent, while in 2 others poorly formed, distorted tubules were observed. In the tubular types there were many cells lying free in the lumina; the nuclei were basal in cells at the periphery of the tubules. The nuclei in the tubular types contained much chromatin, which was not so extensive in the diffuse types. In all cases large nucleoli were prominent in the cells.

*The tumor lipids.*—In normal adult dog and human testis stained for sudanophilic material (fat), large quantities of lipid were found in the interstitial cells of Leydig and in the Sertoli cells. The germinal epithelium was practically fat-free. All the estrogen-producing tumors contained intracellular sudanophilic material, usually in a single large globule; in the cells of interstitial cell tumors it was distributed diffusely in many small droplets. The total lipid content of the tumors was 35.7 to 75.1 gm. per kgm. of fresh tissue; the average value for normal canine testis is 20.3 gm. per kgm. (16).

*Tumor estrogens.*—Two of the testicular tumors from feminized dogs were extracted for estrogen and values of 3.4 and 70 μgm. per kgm. expressed in terms

[1] We are indebted to Professor P. P. H. De Bruyn for assistance in the preparation of the gelatin sections, and to Dr. William W. Scott for aid in the estrogen assays. Dr. Erwin Schwenk, of Schering Corporation, generously furnished prolactin and estradiol.

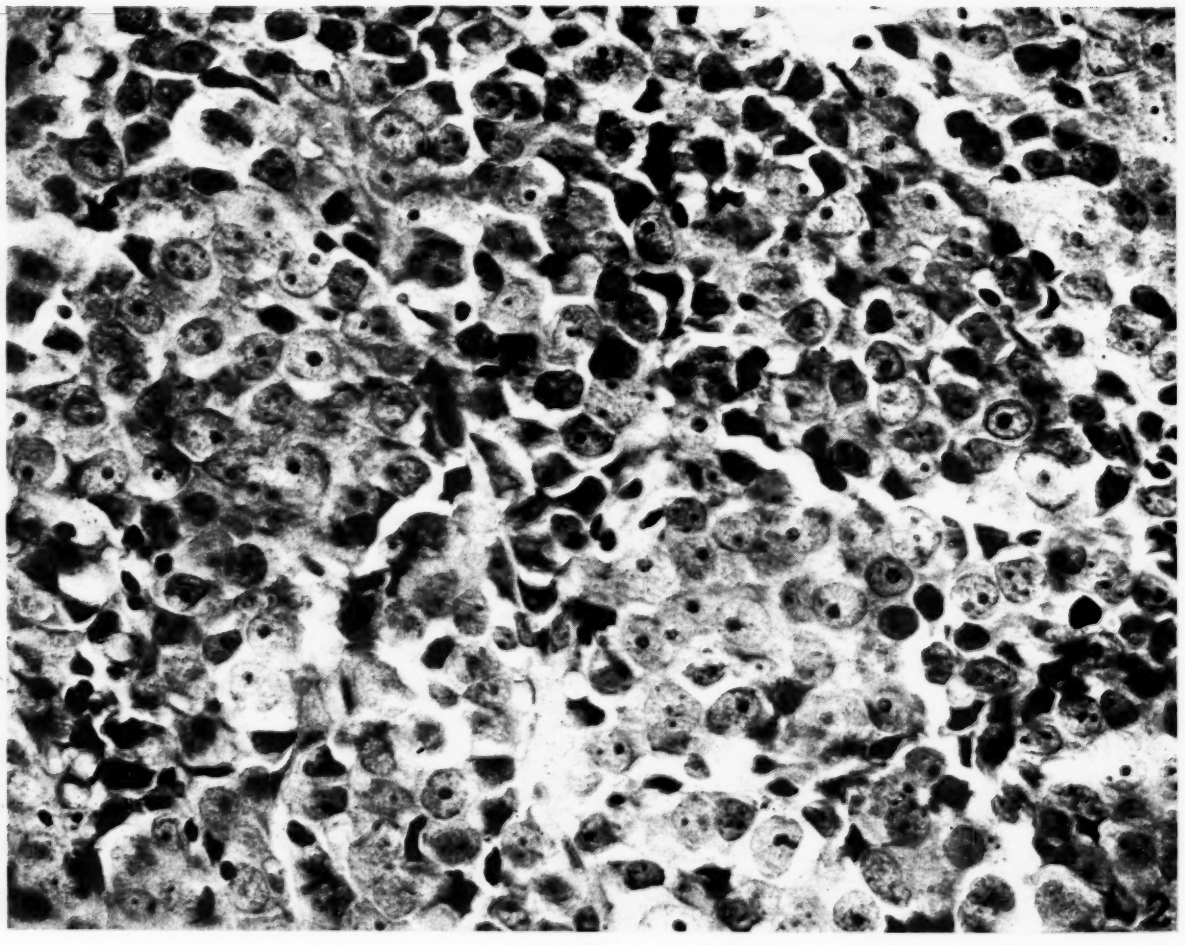

FIG. 1.—Hypertrophy of mammary papillae in a dog with a Sertoli cell tumor in a cryptorchid testis.
FIG. 2.—Sertoli tumor of diffuse type from the testis of a feminized dog. Mag. × 475.

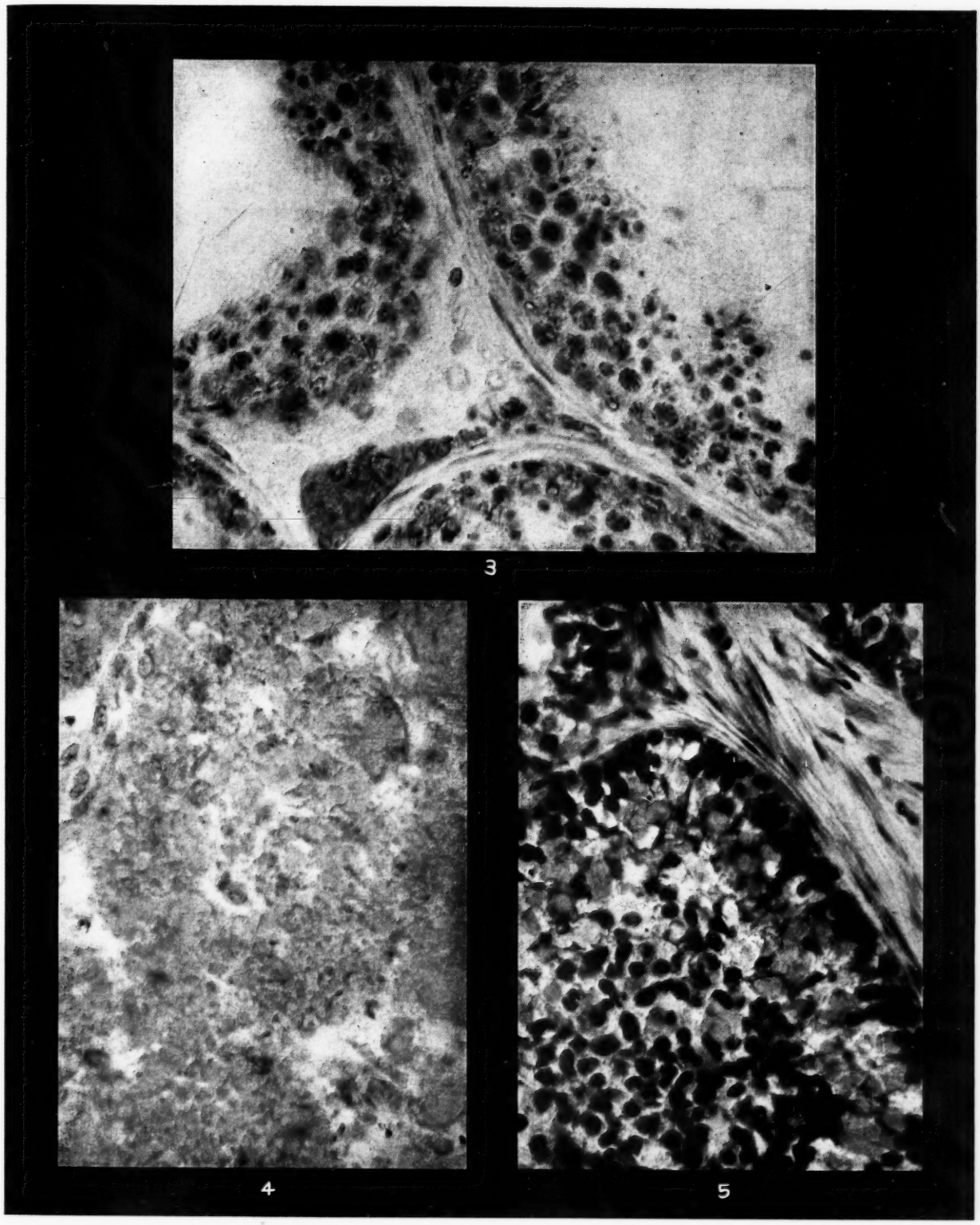


FIG. 3.—Fat-filled interstitial and Sertoli cells of normal human testis.

FIG. 4.—Interstitial cell tumor of canine testis stained for fat.

FIG. 5.—Sertoli cell tumor of tubular type of canine testis stained for fat. The similarity of the cells in the lumen of the larger tubule, and the cells of Fig. 2, may be noted. All of these cytologic preparations were stained with Sudan 3 and Delafield's hematoxylin. Mag. × 300.

of α-estradiol benzoate, were obtained. Estrogen could not be extracted from a Sertoli cell tumor that had not caused feminization. Combined ovaries weighing 13.7 gm. from 5 dogs at estrus (vaginal bleeding) contained the estrogen equivalent of 34 μgm. per kgm.

## DISCUSSION

The site of estrogen production in the testis has not been identified previously. The germinal epithelium of the gland is certainly not its source: The tumors derived from germinal epithelium (seminomas) do not cause squamous metaplasia of the prostate (17), and femaleness occurred in a male dog with bilateral cryptorchism with associated testicular tumors in the present series. While it is readily feasible to eliminate the germinal cells by cryptorchism or administration of certain steroids (androgen or estrogen) no experimental approach has been devised to eliminate selectively the interstitial apparatus or the Sertoli cells. The overgrowth of a specialized cell in a tumorous process provides an opportunity to study the physiologic effect of overgrowth of a single cellular type.

Evidence advanced previously regarding the testicular origin of estrogen is inconclusive; both Sertoli and Leydig cells have been implicated, but the evidence has been inferential rather than substantive.

In a human hermaphrodite with breast hypertrophy Witschi and Mengert (31) found an abundance of Sertoli cells in abdominal testes, to which they ascribed the production of estrogen; estrogen was excreted in the urine and it disappeared after castration. In this testis clusters of the interstitial cells of Leydig were present, though they were small and did "not give the impression of much activity." These authors point out the morphologic homology of ovarian granulosa and sustentacular cells of the testis, stating that both arise from the follicle cells of the primordial gonia.

Evidence that the Leydig cell produces estrogen is derived from the assumption that certain tumors of the human testis associated with mammary hyperplasia are interstitial cell growths (18, 23, 24). This interpretation does not fit in with other cases where interstitial cell neoplasms produced large amounts of the male sex hormones. In children several tumors of the testes that cytologically resembled interstitial cell growths of the dog have been described (27, 29); they effected precocious growth of the sexual organs and muscular development—clear evidence of androgen production. An adult described by Masson (22) with malignant interstitial cell carcinoma had an extremely high titer of urinary androgen but no gynecomastia; extracts of the urine of this man contained 980 to 1,040 mgm. of 17-ketosteroids *per diem* (30) in the ketonic fraction.

It is now possible to prove that the estrogen-producing tumors of the testis are derived from Sertoli cells; the evidence is based on histochemical study of the lipids and on estrogen extraction and assay.

Sertoli (28), in his description of the sustentacular cells of the tubules of the testis, stated that they contain fat. By histological methods the lipids (11) of the testis were found present normally in 2 locations: the sustentacular and the interstitial cells. The germinal epithelium is nearly fat-free.

All the testicular tumors of feminized dogs contained large amounts of lipid; from 75 to 370 per cent more fat than normal canine testis. In 3 the neoplastic cells were arranged in tubular formation while in 2 others tubular formation was present, but the tubules were abortive and transition stages were traced between strictly tubular and diffuse patterns of the same growth. By definition the cells of the testicular tubules with high lipid content are sustentacular cells.

The estrogen content of these lipid-rich neoplasms is considerable; in 1 case it was equivalent on bioassay to 0.07 mgm. per kgm., calculated as α-estradiol benzoate, being twice the content of the ovaries at estrus and one-third of the amount in equine testis, the richest known source of estrogen.

Not all the Sertoli cell tumors of the testis produce estrogen in sufficient quantity to exert a physiological effect. With respect to the prostate of the castrate dog, androgen in adequate amount is able to mask the action of injected estrogen; specifically testosterone propionate, 10 mgm., is able to mask the effects of diethylstilbestrol in amounts up to 0.4 mgm. (15). Therefore unless the amount of androgen produced is exceeded by estrogen in fixed ratio the feminizing effects of estrogen on the prostate are concealed.

Do the Leydig cells produce estrogen? Since the observations of Bouin and Ancel (2) most workers have agreed that androgen is produced in the interstitial cells of the testis. It would be surprising if physiologically antagonistic substances such as androgen and estrogen were produced in the same cell. A close cytologic similarity exists between the diffuse Sertoli cell tumors of the canine testis (Fig. 2) and those human tumors associated with mammary growth and previously classified as interstitial cell tumors. The interstitial cell growths of the dog are characteristic in their extratubular location and highly vacuolated appearance; invariably they are associated with tall cylindrical prostatic epithelium, often hyperplastic and cystic (evidence of androgen dominance), and squamous metaplasia is lacking. The cytologic appearance is quite different from the diffuse Sertoliform tumors, where diffuse vacuolation is not observed.

Difficulty exists concerning nomenclature of the feminizing neoplasms of the testis: the term tubular

adenoma is unsatisfactory, since in certain cases the tubules are vestigial or absent; for the same reason the term adenocarcinoma (34) is inexact, and none of these tumors, furthermore, has been found to metastasize in dog or man. These difficulties may be eliminated by the use of the terms tubular or diffuse Sertoli cell tumor.

## CONCLUSIONS

Feminizing tumors of the testis are rich in lipids and are growths of the sustentacular cells of Sertoli; tubular and diffuse types and intergrades between these kinds are described. Their estrogen content is considerable; in one case, equivalent to 0.07 mgm. of α-estradiol benzoate per kgm. of fresh tissue—an amount twice as great as in ovaries containing large follicles at estrus.

The cells of Sertoli in the testicular tubules produce estrogen.

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# Induction and Prevention of Fibromyoepithelioma of the Utricular Bed in Male Guinea Pigs

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## INTRODUCTION

Tumors of variable size and form have been discovered in the prostatic region of castrated male guinea pigs to which estradiol dipropionate has been administered for 9 months (19). The tumors were found just beneath the site where the vasa deferentia emerge between the lateral prostatic lobes (Figs. 1 and 2); when of greater size they may encircle the proximal end of the vasa deferentia and the bases of the seminal vesicles (Figs. 4, 5 and 9A). It was thought that they might offer a new opportunity to study various questions related to the control of tumorous growth by steroid hormones.

The peritoneum of the guinea pig responds with fibrous growth to a prolonged administration of estrogens (4, 11). Was the new type of tumor mentioned nothing but a local manifestation of a similar peritoneal response? Or was it to be related to atypical proliferation of the various tissues of the prostatic region? As shown by the pioneer work of Lacassagne, de Jongh, Burrows and Kennaway, von Wagenen, Zuckerman, and many others (see the reviews of Allen and of Zuckerman) an atypical proliferation of both the fibromuscular elements of the utricular bed and the epithelial elements of the prostate and its accessory organs takes place under the influence of estrogens. There are considerable species differences as to the parts or organs involved; but squamous stratified metaplasia of the utriculus has been described also in the guinea pig (2, 6, 17). Evidence will be given in this paper that the tumor is a preutricular fibromyoma invaded by epithelial cords from the utricular mucosa and the vasa deferentia.

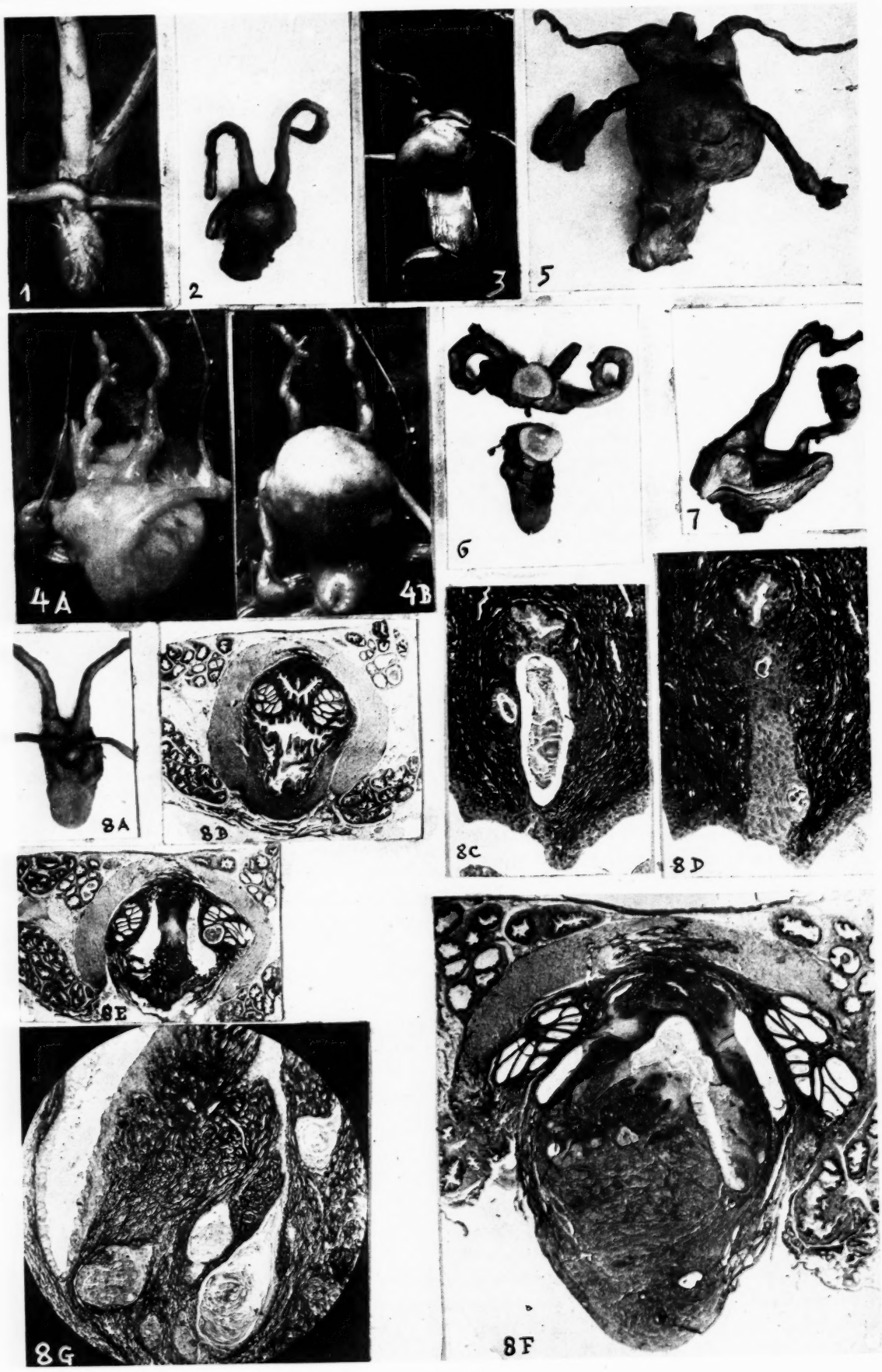
It has been found by Zuckerman (21) and by Zuckerman and Parkes (22) that the fibromuscular proliferation of the utricular bed and the metaplasia of the utricular mucosa in the macacus can be prevented when testosterone propionate is administered simultaneously with the estrogen. Similar statements have been made for mice (16) and rats (18). With progesterone prevention was not obtained in the macacus (22). On the other hand, it has been shown by our work that estrogen-induced uterine and abdominal fibroids can be prevented in the guinea pig by these and other steroids (7, 9, 15). Regression of already elicited fibroids was obtained also by the subsequent administration of progesterone (12, 13). The question arose whether prevention and regression of the estrogen-induced fibromyoepithelioma of the utricular bed of the guinea pig would also be possible.

## FREQUENCY OF FIBROMYOEPITHELIOMA OF THE UTRICULAR BED

Results with 121 *castrated* male guinea pigs are summarized in Table I. Tumors visible to the naked eye were present in 42 per cent of animals subjected to the action of estrogens for 7 to 11 months. But tumors may appear earlier; there was one case with a tumor at 108 days.

It must be held in mind that our figures of frequency—11.5 per cent at 3½ to 6 months, 42 per cent at 7 to 11 months—refer to tumors visible to the naked eye and not to the reaction of the utricular region as described by former authors.

A group of 23 *intact* male guinea pigs was subjected to the action of subcutaneously implanted tablets of stilbestrol for 6½ to 16½ months. There were 4 cases with tumors. In a group of 6 uncastrated males in which the experiment lasted 4 to 6 months a large tumor was found 179 days after implantation of the stilbestrol tablet. Whereas these experiments show conclusively that the tumor can be elicited in the presence of the testicle, and probably as early as in castrated males, nothing definite can be said on the question whether the testicular androgens were able to diminish the frequency of the tumor. It is known that utricular metaplasia in the intact guinea pig is less readily elicited by estrogens than in castrates (2); this applies also to the rat (18). In the present experiments with uncastrated males the incidence of tumors was indeed less than in castrates; but the total number




FIGS. 1-8G.

of uncastrated males was small, and one might question the significance of the difference.

### Structural Characteristics of the Preutricular Fibromyoma

Forty-three tumors have been observed, of which 25 have been studied microscopically.

In a transverse section of the tumor, spaces can be seen that are due to an enlargement of the proliferating utriculus or of the vasa deferentia; sometimes they are cystic (Figs. 9B and 13A). The cysts are embedded in fibromuscular tissue. When spaces are absent the whole tumor consists of fibromuscular tissue (Figs. 8F, 14B, and 14C) with a varying number of epithelial cords enclosed in it.

The fibromyoma, covered on its ventral aspect by the peritoneum, is encapsulated by fibrous tissue (Fig. 14C). Dorsally the limit is not sharp, especially when there is an invasion by the proliferating utricular epithelium. This invasion occurs in most cases (Figs. 8F and 14B). The ventral peripheral layers are in general purely muscular, with a small admixture of connective tissue fibers between the bundles of smooth muscle cells (Figs. 12B and 14C). The nearer to the center the greater the quantity of sclerotic tissue. Central infiltration with small cells, and necrosis, may occur also (Figs. 12C and 12D).

The description here given makes it evident that the estrogen-induced preutricular fibromyoma is structurally different from the uterine or abdominal fibroid

Table 1: One Hundred and Twenty-One Castrated Male Guinea Pigs with Subcutaneously Implanted Tablets of Various Natural and Artificial Estrogens *

| Groups | Estrogen | Estrogen absorbed per day, μgm.† | Duration of experiment, days | Number of animals: Total | Number of animals: With tumors of the utricular bed | Animals with tumors: Estrogen absorbed per day, μgm. | Animals with tumors: Duration of the experiment, days |
|---|---|---|---|---|---|---|---|
| I | Estradiol dipropionate | 12–31‡ | 105–160 | 17 | 0 | ..... | ..... |
| II | Estradiol dipropionate | 13–55‡ | 204–331 | 40 | 19 | 12–37 | 275–330 |
| III | α-Estradiol | 36–52 | 207–286 | 6 | 2 | 44–46 | 286 |
| IV | Stilbestrol§ | 4–15 | 108–182 | 33 | 6 | 4–15 | 108 (8μgm.)<br>147–182 |
| V | Stilbestrol§ | 6–18 | 207–342 | 14 | 4 | 9–13 | 207–342 |
| VI | Hexestrol§ | 3–7 | 142–170 | 11 | 1 | 3 | 142 |
| Summary: | All estrogens | | 105–182 | 61 | 7 | 11.5% | |
| | All estrogens | | 204–342 | 60 | 25 | 42.0% | |

* Observations of Drs. R. Iglesias, T. Monreal, and M. Echániz also included.
† Total absorption divided by number of days.
‡ Calculated as free α-estradiol.
§ Absorbed from tablets containing 40 percent of stilbestrol or hexestrol and 60 percent of cholesterol.

## DESCRIPTION OF FIGURES 1 TO 8G

Fig. 1.—Normal adult guinea pig. Seminal vesicles, vasa deferentia, urinary bladder; prostatic lobes lateral to seminal vesicles.

Fig. 2.—Tumor at confluence of vasa deferentia. Diethylstilbestrol, 164 days; 9 μgm. per day.

Fig. 3.—Estradiol dipropionate, 277 days; 15 μgm. of α-estradiol per day.

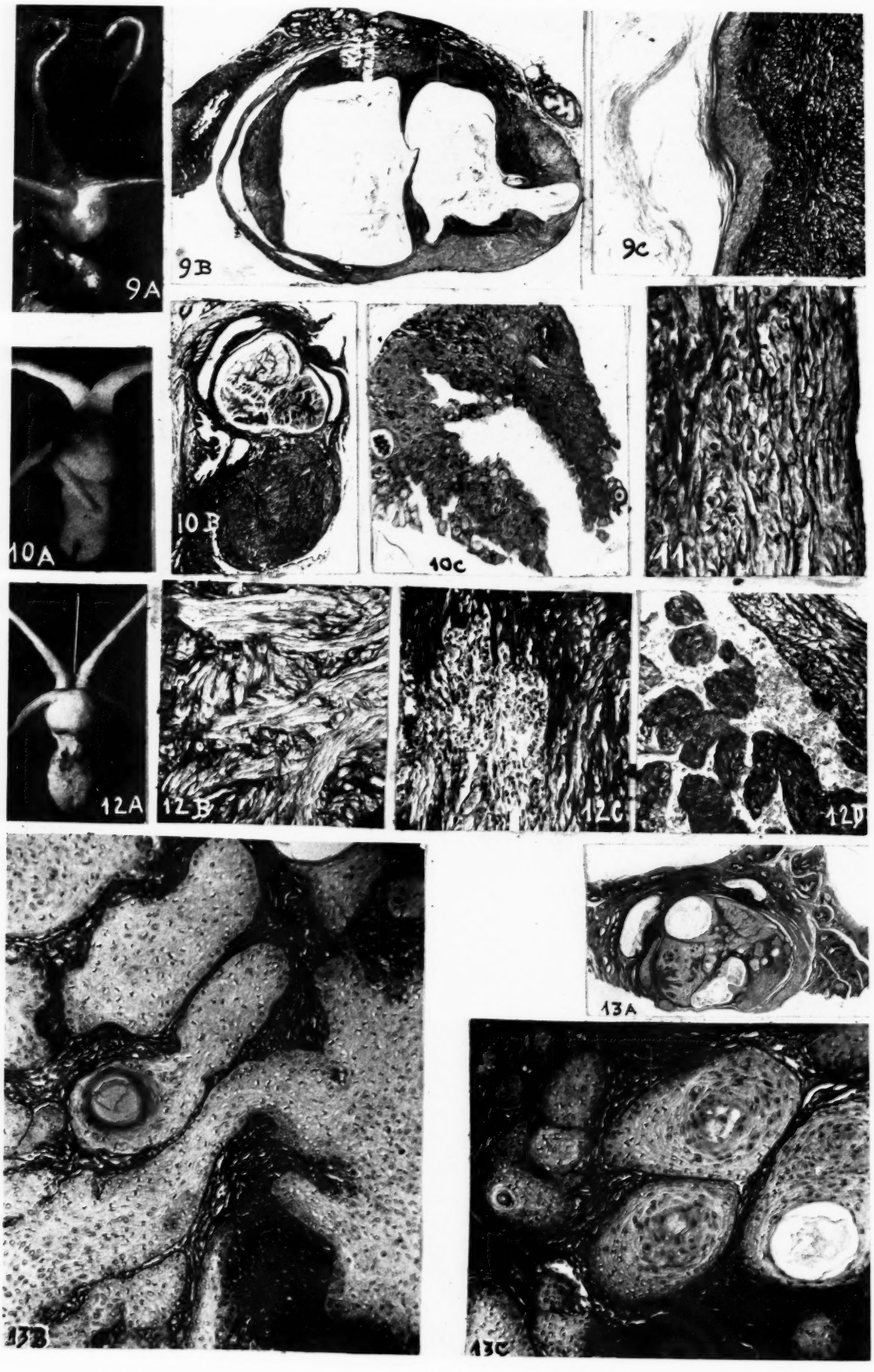
Fig. 4.—Enormous fibromyoma. Estradiol dipropionate, 275 days; 12 μgm. of α-estradiol per day. A. Left vas deferens embedded in the tumor. B. Tumor raised to show urinary bladder.

Fig. 5.—One of the largest tumors. Estradiol dipropionate, 330 days; 13 μgm. of α-estradiol per day. Uneven surface of tumor. Two additional tumors around seminal vesicles. Urinary bladder and penis beneath the large tumor. Note also estrogen-induced fibroid of right vas deferens, at site where vas deferens has been cut for castration ("inguinal" tumor).

Fig. 6.—Transverse section of tumor. Estradiol dipropionate, 279 days; 15 μgm. of α-estradiol per day.

Fig. 7.—Sagittal section of tumor. Estradiol dipropionate, 275 days, 22 μgm. of α-estradiol per day. Inguinal tumor of left vas deferens.

Fig. 8.—A. Small tumor. Diethylstilbestrol, 108 days; 8 μgm. per day. B to G. The same animal as A. B. Transverse section of caudal end of prostatic region, somewhat above the verumontanum. Prostatic acini around the circular muscle. Ventrally the urethral diverticulum is seen uniting with the urethra, and dorsally the confluence of both ejaculatory ducts. Note tiny utriculus dorsal to the ejaculatory ducts. To both sides of the latter, prostatic ducts. Appearance normal. Mag. × 5. C. Utriculus as seen in B, but at mag. × 90. Epithelium flattened. D. Utriculus at about 0.3 mm. above B and C; utriculus transformed into a solid epithelial cord. Mag. × 90. E. About 0.3 mm. above D. Utriculus makes contact with ejaculatory ducts and branches also ventrally. Mag. × 5. F. About 0.8 mm. above E. Greatly enlarged utricular cavity; epithelial proliferations penetrating deeply into ventral fibromyoma. Lateral to enlarged utriculus, vasa deferentia transformed into solid epithelial cords not yet separated from the ejaculatory ducts. Mag. × 10. G. Detail from section about 0.7 mm. above E. Deep penetration of epithelial cords into fibromyoma. Cornification of proliferating buds that may become necrotic. Mag. × 45.




FIGS. 9A-13C

induced by estrogens in the guinea pig. In the fibroid of peritoneal origin cells rich in protoplasm, probably fibroblasts, predominate; smooth muscle fibers, if present at all, are not very numerous (14). On the contrary, smooth muscle fibers predominate in the tumor of the utricular bed. This structural difference, indicating a fundamental difference of origin of the two types of fibromyoma, can best be exemplified by cases in which an additional tumor of peritoneal origin around the seminal vesicle is present besides the preutricular tumor (Figs. 5 and 11).

### Epithelial Proliferation

Extensive work has been done on the estrogen-induced metaplasia of the utricular mucosa in the male of different species. This metaplasia is identical with the condition of the vaginal mucosa in estrus (2, 20, 21). It is maintained that the epidermization of the utricular mucosa starts at its caudal end, and that the same occurs with respect to the vasa deferentia. Our own results in the guinea pig are not in full agreement with this statement as to the utriculus. In sections of the extreme caudal end of the utriculus in animals with a preutricular fibromyoma nothing abnormal was seen. The mucosa of the utriculus in the guinea pig is identical with that of the urethral cephalic diverticulum into which the utriculus opens. It consists of 1 to 2 layers of cuboidal cells. The utricular epithelium may be flattened sometimes (Fig. 8c). Abundant proliferation of the epithelium can be seen in sections at a certain distance from the caudal end (Fig. 8d); the utriculus may be transformed into a solid mass of epithelial cells. Further cephalad the proliferation becomes more extensive (Fig. 8e). Epithelial digitations are formed, which may attain considerable length and which penetrate deeply into the fibromyoma (Fig. 8f). In the latter an abundance of epithelial cords with cornification or vacuolization of the superficial layers is to be seen (Fig. 8g). The epithelial cords often become necrotic. Sometimes there is an overwhelming mass of solid epithelial cords in the fibromyoma (Fig. 13).

As already mentioned, the tumor may contain multiple cysts. The walls of these are covered by squamous stratified epithelium with cornification of the superficial layers (Fig. 9c). They may also be filled with papillomatous growths (Figs. 10b and 10c).

The mucosa of the ejaculatory ducts does not show metaplastic changes. These appear further up, where the vasa deferentia separate (Fig. 8f). This metaplasia is identical with that in the utriculus. Further up the vas deferens metaplasia is again absent. The mucosa of the ducts of the seminal vesicles very rarely shows proliferation, and then it is inconspicuous. In the collecting ducts of the prostate and in the prostate itself we have never seen metaplasia.

It is evident that the growth visible to the naked eye evolves from the hyperplastic and metaplastic changes of the male accessory organs of the prostatic region, so well described by various workers during the past ten years. It would be idle to speculate on why these tumors have not been seen formerly. The extension of hyperplasia and metaplasia might depend upon species differences, and the guinea pig has not been favored by investigators in this field. But the most probable explanation is that the evolution of a tumor from these localized hyperplastic and metaplastic changes depends upon the time that the estrogenic stimulus is allowed to act. Localized, or "insular," proliferation as the starting point of neoplastic growth (8) is less striking with reference to the fibromyomatous part of the preutricular tumor. It seems that the neoplastic evolution involves the utricular fibromuscular bed as a whole. But the normal area of the preutricular fibromuscular tissue in the guinea pig is so inconspicuous that its transformation into a

### Description of Figures 9 to 13c

Fig. 9.—a. Estradiol dipropionate, 277 days; 18 μgm. of α-estradiol per day. b. Same tumor, which consists mostly of large cysts embedded in fibromuscular tissue. On left side of tumor the cyst almost reaches the surface. Mag. × 5. c. Same tumor. Wall of cyst cornified. Mag. × 45.

Fig. 10.—Estradiol dipropionate, 290 days; 20 μgm. of α-estradiol per day. a. Irregular form of tumor; deviation of left vas deferens. b. Transverse section of same tumor. Utricular cyst lying outside fibromyoma and filled with papillomatous growth. Mag. × 5. c. Pseudostratified epithelium of papilloma undergoing vacuolization. Mag. × 100.

Fig. 11.—α-estradiol, 286 days; 46 μgm. per day. Subserous tumor at base of seminal vesicle. Typical structure of estrogen-induced fibroid of peritoneal origin; abundant fibroblasts at periphery. Mag. × 200. The structure is fundamentally different from that of fibromyoma of utricular bed. Compare with Fig. 12b and especially Fig. 14c.

Fig. 12.—a. α-estradiol, 286 days; 44 μgm. per day. b. Peripheral part of same tumor; almost exclusively smooth muscle bundles. Mag. × 100. c. Center of same tumor; abundance of connective tissue fibers and small cells. Mag. × 100. d. Other part of center of tumor; cavity filled with detritus and hyalinized masses. Mag. × 100.

Fig. 13.—a. Estradiol dipropionate, 280 days; 15 μgm. of α-estradiol per day. Tumor consists mostly of solid epithelial cords and some cysts embedded in fibrous tissue. Peripheral part myomatous. Laterally, ducts of seminal vesicles. Vasa deferentia cannot be recognized. Mag. × 5. b. Same tumor. Epithelial cords of irregular shape. Mag. × 90. c. Concentric disposition of cells in epithelial cords of this tumor. Mag. × 90.

neoplasm, as exemplified by Figs. 4 and 5, or even by Figs. 8F, 10B, and 14B, is by itself highly impressive from the point of view of the neoplastic potentialities of a very limited area of cells.

### Simultaneous Action of Estrogens and Antifibromatogenic Steroids

Results with three different antifibromatogenic steroids are summarized in Table II. When the antifibromatogen began acting about 3 to 6 weeks after the estrogen no tumors visible to the naked eye were found. There was only one exception—an animal belonging to the testosterone group, in which total absorption of the tablet had taken place before necropsy was made.

The result was different when tablets of antifibromatogens were implanted about 6 months after the estrogen. Tumors were found in 5 of 37 animals. The difference between both groups is striking. How is this unexpected result to be interpreted?

Tumors of the utricular bed appeared with an incidence of 11.5 per cent after an estrogenic action of 3½ to 6 months (Table I). Very close to this figure was the incidence, 13.5 per cent, in the group of 37 animals with late action of the antifibromatogens (Table II). This coincidence would suggest that the tumors found in the group of 37 animals were already present when the antifibromatogen was added.

Our experiments show conclusively that the three antifibromatogenic steroids were capable of preventing estrogen-induced fibromyomas of the utricular bed, in the same way as they prevent formation of peritoneal fibroids. Further growth of the preutricular fibromyomas also seemed to be stopped, as suggested by the fact that there was no large tumor in the 5 tumor animals mentioned above (the largest is shown in Fig. 17A). One might tentatively infer that progesterone and desoxycorticosterone acetate are unable to cause regression of tumors already elicited. But the preutricular fibromyomas may have persisted in spite of antifibromatogens because the quantities absorbed were too small (Table III). They were equal to, and sometimes even greater than, those that were able to prevent peritoneal fibroids (3, 5, 7, 9, 10), to be sure. But as shown above there are pronounced structural differences between the two types of estrogen-induced mesodermal tumors; *i. e.,* the abdominal fibroid of peritoneal origin and the fibromyoma of the utricular bed. One might ask, then, whether there are quantitative differences of response to antifibromatogenic steroids between these two types of tissues or experimental tumors.

Table II: Sixty-Three Castrated Male Guinea Pigs with Subcutaneously Implanted Tablets of Various Estrogens and Antifibromatogenic Steroids Active for Different Lengths of Time

| Groups | Estrogen | | | Antifibromatogen | | | Number of animals | |
|---|---|---|---|---|---|---|---|---|
| | | Estrogen absorbed per day, µgm. | Estrogen acted, days | | Antifibromatogen absorbed per day, µgm. | Antifibromatogen acted, days | Total | With tumors of utricular bed |
| Ia | Estradiol dipropionate | 20–33 | 200–315 | Progesterone | 10–26 | 175–290 | 6 | 0 |
| Ib | Stilbestrol | 4–17 | 302–319 | Progesterone | 5–19 | 261–278 | 8 | 0 |
| Ic | Estradiol dipropionate | 23–36 | 295–299 | Progesterone | 20–44 | 109–113 | 7 | 2 |
| Id | Stilbestrol | 3–13 | 306–313 | Progesterone | 15–105 | 112–119 | 9 | 1 |
| IIa | Stilbestrol | 11–33 | 220–303 | Desoxycorticosterone acetate | 153–236 | 199–282 | 6 | 0 |
| IIb | Stilbestrol | 5–31 | 298 | Desoxycorticosterone acetate | 169–190 | 112 | 8 | 0 |
| IIc | α-Estradiol | 27–45 | 283–287 | Desoxycorticosterone acetate | 156–180 | 138 | 7 | 2 |
| IIIa | Estradiol dipropionate | 20–40 | 317 | Testosterone propionate | 162–184 | 294 | 6 | 1* |
| IIIb | Estradiol dipropionate | 28–35 | 300 | Testosterone propionate | 120–506 | 117 | 6 | 0 |
| Summary: | All estrogens | | | All antifibromatogens | | Starting 3 to 6 weeks after estrogen | 25 | 0 |
| | All estrogens | | | All antifibromatogens | | Starting 5 to 6 months after estrogen | 37 | 5 |

* Absorpotion of testosterone was complete before necropsy. Omitted in summary of this table.

### Structural Characteristics of Preutricular Tumors Present in Spite of Antifibromatogens

Estrogen-induced peritoneal fibroids shrink and become hyalinized when progesterone is added (13). On the contrary, with the late action of the antifibromato-

gen there were no structural changes in the preutricular tumors comparable to those found in peritoneal fibroids under similar experimental conditions. There was partial sclerosis or hyalinization in the center of the tumor of the utricular bed in all 4 tumors examined (Figs. 17B and 18B), but this was found in almost every fibromyoma of the utricular bed, though in varying degree, also in animals in which estrogen alone was acting. More relevant is the observation that in all the fibromyomas of the utricular bed that persisted in spite of antifibromatogens there was a great abundance of smooth muscle bundles in the periphery, seemingly without any sign of degeneration.

Epithelial proliferation was in all respects similar to that found in animals not subject to the simultaneous action of an antifibromatogen. There was papillomatous growth filling the cystically enlarged utriculus or vas deferens (Fig. 18A). There was also squamous stratified epithelium with cornification, concentric disposition of cells, and formation of pearls (Fig. 18B). In one case proliferation of the vacuolated type of epithelium predominated (Figs. 16C an 16D); but this type of cell was also abundant in tumors when estrogen alone was acting. The utriculus at a certain distance above the verumontanum offered the characteristic aspect of atypical growth (Fig. 16B). Persistence of epithelial proliferation in spite of antifibromatogens could readily be explained on the same quantitative basis as persistence of the myomatous part of the tumor.

## SUMMARY

Tumorous growths attaining in some cases a diameter of 2 cm. or more were elicited in the prostatic region of male guinea pigs by the prolonged action of various estrogens—alpha-estradiol, estradiol dipropionate, stilbestrol, and hexestrol—absorbed from subcutaneously implanted tablets. The earliest tumor appeared 108 days after their implantation. Incidence increased greatly with the greater length of estrogenic action. In experiments lasting from 7 to 11 months the incidence reached more than 40 per cent in castrated animals and seemingly less in intact ones.

The tumors were fibromyoepitheliomas, and resulted from further hyperplastic growth of the fibromuscular preutricular tissue and metaplastic growth of the mucosa of the utriculus and a limited area of the vasa deferentia. The fibromyomatous part of the preutricular tumor was structurally different from the estrogen-induced abdominal fibroid of peritoneal origin. Whereas fibroblasts originating from the peritoneum prevailed in the latter, bundles of smooth muscle fibers originating from the utricular bed prevailed in the former.

TABLE III: FIVE CASTRATED MALE GUINEA PIGS WITH TUMORS OF UTRICULAR BED
Late action of progesterone or desoxycorticosterone acetate

| No. of animal | Estrogen alone, | Simultaneous action of estrogen and antifibromatogen, | Average absorption per day | | Microscopical condition of the tumor | |
|---|---|---|---|---|---|---|
| | | | Estrogen, | Antifibromatogen, | Squamous epithelium and cornification | Myomatous tissue |
| | days | days | μgm. | μgm. | | |
| | | | E'diol dipr.* | Progester. | | |
| LIX. 49 | 186 | 109 | 23 | 44 | † | † |
| LIX. 50 | 186 | 109 | 28 | 20 | + | + |
| | | | Stilbest. | | | |
| LIX. 37 | 194 | 119 | 64 | 42 | + | + |
| | | | α-estrad. | Desoxyc. ac.* | | |
| LX. 57 | 145 | 138 | 35 | 156 | 0 | + |
| LX. 51 | 145 | 138 | 40 | 180 | + | + |

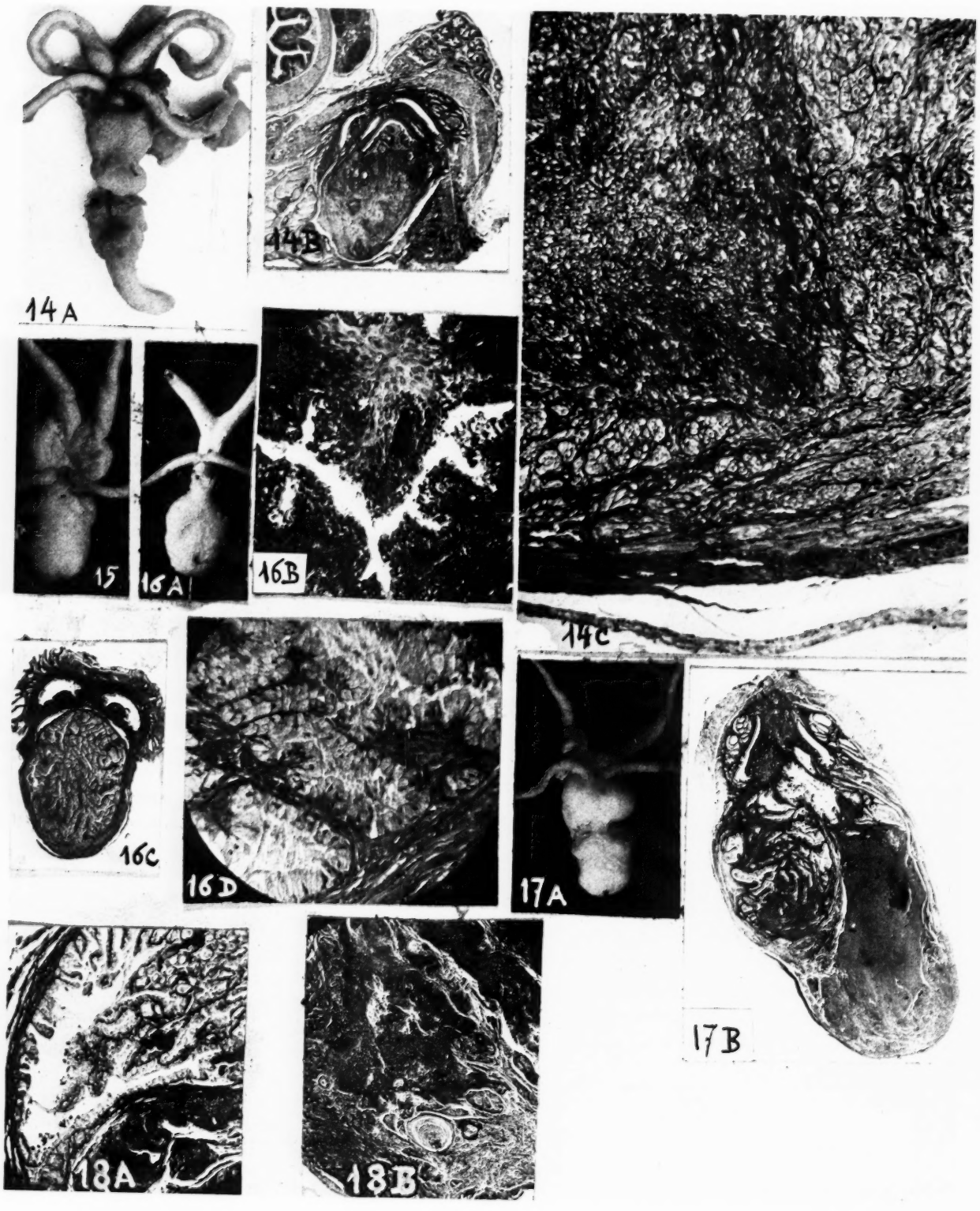
* Calculated as free hormone.
† Not examined.

The proliferative and especially the metaplastic changes of the utricular mucosa started not at its extreme caudal end, but a certain distance cephalad.

The estrogen-induced fibromyoepitheliomas of the utricular bed were prevented when, after a few weeks of action of the subcutaneously implanted estrogenic tablets, tablets of antifibromatogenic steroids such as progesterone, desoxycorticosterone acetate, and testosterone propionate were implanted.

However, when the antifibromatogenic steroids were added 5 to 6 months after the implantation of the estrogenic tablet and allowed to act for another 4 months, fibromyoepitheliomas of the utricular bed were present in a number of cases, and their structure was the same as that of tumors arising from estrogen alone. But the incidence of these tumors was lower and their size smaller than with estrogens only.

The question is discussed whether there are quantitative differences of response to antifibromatogenic steroids between the two types of estrogen-induced mesodermal tumors; *i. e.,* peritoneal fibroid and the preutricular fibromyoma.




FIGS. 14A-18B

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DESCRIPTION OF FIGURES 14A TO 18B

Fig. 14.—A. Small tumor. Estradiol dipropionate, 282 days; 14 μgm. of α-estradiol per day. Behind seminal vesicles, the rectum. "Inguinal" tumor of the left vas deferens. B. Transverse section of same tumor. Fibromyoma with epithelial utricular proliferation. Note also vasa deferentia and ducts of seminal vesicles. Mag. × 5. C. Periphery of same fibromyoma. Abundance of smooth muscle bundles. The nearer to the center the greater the quantity of fibrous tissue. Mag. × 90. Compare structure of this typical fibromyoma of the utricular bed with structure, also typical, of subserous peritoneal fibroid in Fig. 11.

Fig. 15.—Estradiol dipropionate, 30 μgm. of α-estradiol per day. Tablet of testosterone propionate implanted 183 days after estrogen; 145 μgm. of testosterone per day in the course of 117 days. Normal aspect of lateral prostatic lobes. Absence of any prominence beneath vasa deferentia.

Fig. 16.—A. Small tumor. α-estradiol, 35 μgm. per day. Tablet of desoxycorticosterone acetate implanted 145 days after estrogen; 156 μgm. per day in the course of 138 days. B. Transverse section of same tumor, somewhat above verumontanum. Confluence of ejaculatory ducts. Utriculus transformed into solid cord of epithelial cells. Mag. × 100. C. Transverse section of tumor cephalad, which consists of tubular structures and a trabecular system. Laterally, vasa deferentia and ducts of seminal vesicles. Mag. × 5. D. Tubular structures are lined with large vacuolated cells similar to vaginal epithelium during pregnancy. Smooth muscle fibers in trabecular system. Mag. × 100.

Fig. 17.—A. Largest tumor in group of 5 that developed in spite of antifibromatogens. Estradiol dipropionate, 28 μgm. of α-estradiol per day. Tablet of progesterone implanted 186 days after estrogen; 20 μgm. per day in the course of 109 days. B. Transverse section of same tumor. Dorsally, ejaculatory ducts visible, and to both sides of these the prostatic ducts. Cysts and abundant epithelial proliferation deriving from utriculus, especially in right half of tumor (left in the figure). Extensive hyalinization in left half of tumor; but peripheral layers remain myomatous. Mag. × 5.

Fig. 18.—Details from tumor elicited by α-estradiol, 40 μgm. per day. Tablet of desoxycorticosterone acetate implanted 145 days after estrogen; 180 μgm. per day in the course of 138 days. A. Papilloma filling cystic cavity. Mag. × 23. B. Epithelial cord with cornification. Abundant sclerotic tissue amid fibromyoepithelioma. Smooth muscle bundles were abundant in periphery. Mag. × 23.

# Potential Differences in the Skin of Mice during Carcinogenesis

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## I. INTRODUCTION

Studies of potential differences (p.d.) in cancer-resistant and cancer-susceptible mice have been carried out by Burr, Smith, and Strong (3), who stated that evidence had been produced of, first, "a characteristic change in the electrodynamic field of a mouse incident to the development of cancer;" and second, "the presence of a potential gradient between tumor and normal areas of the organism, notably during the early development of the tumor." The tumors were spontaneous mammary cancers in strain A mice (Strong). Similar experiments, begun by Dr. Iball, have been carried out in this Institute with (a) C3H and dba mice producing spontaneous mammary cancers; (b) C3H mice fed with 2,2′-diamino-1,1′-dinaphthyl or with 2,2′-azonaphthalene, which induce liver tumors (in our experiments cholangiomas only were produced in a high proportion of animals); (c) stock mice painted on the back of the neck with a solution of the carcinogen 9,10-dimethyl-1,2-benzanthracene in benzene. No obvious relation was observed between tumor development and p.d. In order to clarify the discrepancy between Burr's result and these preliminary observations it was decided to carry out carefully controlled experiments in which the p.d. would be measured between tumor tissue and neighboring normal tissue on the surface of the skin, the tumors being produced conveniently by painting with the carcinogen 9,10-dimethyl-1,2-benzanthracene.

It seemed the more worthwhile to investigate this problem since studies of p.d.'s in living tissues have been carried out by a number of investigators, and in certain cases regularities have been observed that have been associated with definite biological changes; *e.g.*, by Rogers (16, 17), Kaunitz and Schober (9), Burr, Musselman, Barton, and Kelly (2), Reboul, Rock, and their associates (13-15), Snodgrass, Rock, and Menkin (18), Burrows, Iball, and Roe (5). It was felt that carefully controlled experiments might help to clarify the source and character of these p.d.'s and their significance, if any, for the living organism.

## II. APPARATUS

The measuring instrument employed in these experiments has been described in detail in an earlier paper (5). It is essentially a vacuum tube microvoltmeter having high sensitivity and sufficiently high input impedance. The instrument is little affected by external electrical disturbance, and the only shielding required is provided by placing all the apparatus on a grounded copper sheet.

The potential difference to be measured is applied to the grid of one of the valves in a balanced bridge instrument. Contact to the animal is made via two silver-silver chloride electrodes, and a pipette filled with 0.9 per cent saline connects each electrode to the point on the skin selected for measurement. One electrode is grounded and the sign of the potentials measured is therefore either positive or negative with respect to this reference electrode. The instrument is calibrated by means of a potentiometer and a standard cell. Sensitivities of 70 mv. per mm. deflection, or a tenth or a 100th of this value have been found convenient for the experiments described in this paper.

Nistler and Pekarek (12) have recommended the use of micro-egg-white electrodes for the measurement of potential differences in living tissue. In these electrodes a silver wire coated with silver chloride dips into coagulated egg-white in a capillary tube, of diameter down to 10$\mu$. Pairs of these electrodes were tested for use in this experiment but they were found to be less efficient than the saline pipette silver-silver chloride electrodes formerly employed. Potential differences between a pair of egg-white electrodes were often large and variable; the egg-white gradually deteriorated, so that some electrodes needed replacement after a few days; and it was impossible to obtain reliable contact between these electrodes and the sur-

face of unwetted skin or of skin rubbed with direct-contact electrode jelly. Saline silver-silver chloride electrodes were used therefore throughout this experiment. The tips of the saline pipettes were drawn out to about 1 mm. diameter, and in order to confine the region of measurement as closely as possible the diameter of the area of skin moistened with saline was normally about 5 mm.

In order to insure that the position of the measuring points on the skin of the animal was standardized as far as possible for consecutive measurements and for different animals, the 2 saline pipettes in their ebonite insulators were clamped side by side in a metal holder so that the tips of the pipettes were always 18 mm. apart and equidistant from the midline of the animal's back. Steel rods projecting downwards from the metal holder served to determine the placing of the electrodes about the midline of the back. The animal was arranged so that one rod touched the point of junction of the tail with the body (a well defined reference point), and the other was arranged over the spinal column at a constant distance from the first.

### III. EXPERIMENTAL PROCEDURE

Thirty-six male MRC mice (obtained from the Medical Research Council; inbred but not brother-sister mated) aged 80 to 105 days were used in this experiment, and the p.d. was measured twice weekly in each animal for a maximum period of 23 weeks. One pair of measuring points was used throughout, *i.e.,* on the skin of the back over the scapulae, at a distance of 9 mm. either side of the midline of the back and 4 cm. from the tail junction. For the measurement of p.d. the animal was immobilized on a cork board by means of elastic bands attached to the paws, and the hair was clipped to the skin at the measuring positions. Anesthesia was found to be unnecessary. The skin was moistened with saline at these positions and the electrode pipettes were lowered into the salt pools. The p.d.'s were recorded between the area to be treated and the unpainted area over the opposite scapula on which the grounded electrode was placed, and which therefore was used as a reference point. In all animals measurements were made for a fortnight before painting was commenced (*i.e.*, five readings) in order to determine the normal limits of variation of the p.d. After this preliminary period an area of skin over one scapula was painted with the solution of the carcinogen in acetone, or with acetone alone, immediately following each potential measurement in each animal. For the purposes of painting and measurement of potentials the animals were divided into 3 groups: (a) 15 animals painted over the left scapula twice weekly with 0.05 cc. of 0.1 per cent 9,10-dimethyl-1,2-benzanthracene in acetone: *i.e.*, receiving 50$\gamma$ of the carcinogen at each application; (b) 11 animals treated similarly on the right side of the back; (c) 10 animals used as controls and painted with the same volume of acetone twice weekly, 5 over the left and 5 over the right scapula. The liquids were applied by means of micro-pipettes, and evaporation was hastened and the area of treatment confined by gentle blowing. For 2 weeks at the end of April (*i.e.*, for 4 p.d. measurements) painting and measurements were unavoidably discontinued, and owing to the death of 8 animals and the weakness of a number of others after about 70 days' treatment, painting was discontinued for a further period (May 6th to June 3rd; *i.e.*, 8 p.d. measurements) in all animals, and was recommenced when the animals had recovered. Measurements of p.d.'s were continued throughout this latter period.

### IV. HISTOLOGICAL RESULTS

The histological changes brought about in the skins of these mice by the applications of 9,10-dimethyl-1,2-benzanthracene provided no new features. The first obvious effect of the painting was epilation. At first the lost hair was renewed; with continued applications the local baldness persisted, and was associated with a disappearance of hair follicles and sebaceous glands. When this stage had been reached there was a diffuse epidermal hyperplasia in the treated area. Normally the epidermis of the mouse's back, where the applications were made in this experiment, consists of a single or at the most a double layer of noncornified cells covered by a thin keratinous coat. The nuclei of these noncornified epidermal cells are small, and become deeply stained by hematoxylin. In the hyperplastic stage induced by the carcinogen the epidermis became thickened, and consisted of 2 or more layers of swollen, noncornified cells with large faintly staining nuclei, and the overlying coat of keratinized cells was sometimes thicker than normal. This epidermal hyperplasia extended for a short distance only along any remaining hair follicles.

After these changes had become established papillomas arose, and the epithelial ingrowths from these sometimes gave the impression that they represented hair follicles.

The transition from papilloma to cancer was not shown by any pronounced and obvious cytological change. The rather crude criterion we have used to distinguish a papilloma from a cancer has been the relationship between the epithelial ingrowth and the panniculus carnosus. If the latter had become invaded by the epithelium the tumor was classified as malignant. This method of distinguishing a malignant from

an innocent growth, though arbitrary, has been used by many to determine the malignancy or innocence of tumors in animals, and practical experience seems to have established its utility.

Among the less regular reactions to carcinogen seen in our mice have been:

(a) The appearance of deeply pigmented maculae where the carcinogen had been applied. The pigment resembled melanin, was deposited thickly in cells in the dermis, and the deposits were not accompanied by cellular proliferation. This response occurred in 2 of the mice.

(b) Another unusual occurrence has been the development of 2 different types of tumor adjacent to each other. In each of the 2 instances in which this occurred there existed a cornifying carcinoma on the surface underlain by a mass of invasive cells resembling lymphocytes.

## V. DESCRIPTION AND RESULTS OF STATISTICAL TESTS

Our results showed no obvious correlation between p.d.'s and changes in the skin during carcinogenesis. They have therefore been treated statistically.

### A. Uniformity Trial Preceding Experiment

Five measurements on each animal were made prior to any treatment apart from clipping the hair at the measuring positions. These measurements were analyzed as a uniformity trial in order to discern any unusual characteristics in the p.d. of these animals. Particular attention was paid to any asymmetry in the p.d. of the left versus right side of the animal. Pooling these 5 measurements for all 36 animals ($n=180$), it is calculated that the mean of their distribution ($\bar{x}$) is $+0.444$ millivolt (left side positive with respect to the right side), the standard deviation ($\sigma$) is 2.920, and the standard error is 0.217. Thus this $\bar{x}$ is significantly different from zero. We have therefore corrected all mean p.d.'s to this zero ($+0.444$ mv.) unless otherwise stated.

### B. Tests on Single Animals

Statistical tests were then made in order to discern whether carcinogenesis had any effect on the p.d. In the subsequent tests, therefore, the p.d.'s were taken in the sense: painted side versus unpainted side unless otherwise stated. Thus measurements were combined irrespective of the side of painting, since the potential at the painted area was always considered with respect to the grounded electrode at the unpainted area.

In the absence of a definite hypothesis as to the nature of such an effect on p.d.'s, different analyses of variance were carried out in order to probe for any possible relationship. The principle of these analyses was as follows:

From the skin changes observed in each treated animal certain more or less accurately defined stages during carcinogenesis could be marked as time intervals of the total experimental period, and thus the p.d. measurements on each treated animal could be grouped according to classifications 1 to 5 shown below:

1. All measurements of p.d. before the macroscopic detection of a papilloma, tested against the remaining measurements.

2. All measurements before the macroscopic detection of malignancy, tested against the rest (malignancy being detected macroscopically by visual observation and palpation, the feeling of a hard, fixed object beneath the skin, and confirmed histologically after the death of the animal).

3. A group of 5 measurements consisting of some immediately before and some during the early stages of development of a papilloma, tested against the rest.

4. A similar group of 5 measurements consisting of some immediately before and some during the early stages of malignancy, tested against the rest.

5. Groups arranged according to the macroscopic appearance of the skin, *i.e.,* "clinical" groups, viz., no change ($N$), dry ($D$), hypertrophic ($QP$, *i.e.*, doubtful papilloma), papilloma ($P$), doubtful malignancy ($QM$), malignancy ($M$).

6. In order to test the independence of p.d. measurements made at 2 to 3 day intervals, an analysis of variance was carried out for groups of 5 consecutive measurements.

For each classification and for each animal tests were carried out to determine whether the variation of p.d. values in the *same* class was smaller than that of p.d. values in *different* classes. In other words, the analysis of variance was used to detect any intraclass correlation between clinical stages during carcinogenesis and p.d. measurement.

As a control a similar analysis of variance was carried out for the control animals. Here the p.d. measurements were grouped in pseudo-classifications 1 to 5 in which the number of measurements in each group was made equal to the mean number of measurements in the corresponding group of the treated animals.

To test the influence of painting with carcinogen in acetone, or with acetone alone, the analysis of variance was carried out on the following groups:

7. The group of 5 preliminary measurements on the untreated skin, tested against the rest.

8. The 5 preliminary measurements, tested against the rest (from which were subtracted the 8 measurements from May 6th to June 3rd, when painting was discontinued).

9. The 5 preliminary measurements pooled with the 8 measurements (May 6th to June 3rd) and tested against the rest.

The results of these calculations follow in Tables IA and IB, columns 1 to 9, in which the column headings correspond to classifications 1 to 9. Numerical results are given only for significant variations, *e.g.*, a probability of 0.05 corresponds to a value of $P$ between the 5 per cent and 1 per cent level of significance, and lower values of $P$ (0.01, 0.001, etc.) indicate a lower probability that the event could occur by chance, and thus a higher significance for the result; *w* indicates that the variation within the groups is greater than the variation between groups; *n* that the variation between is greater than within groups but has no significance, and — indicates that the corresponding stage did not occur in the animal. Tumor development is classified as:

| | | | | |
|---|---|---|---|---|
| *o* | animals | that | developed | no tumor |
| *p* | " | " | " | a papilloma |
| *m* | " | " | " | a carcinoma |

In a second test we have investigated whether a tendency for long runs of positive or negative potential differences is in any way correlated with the development of a tumor. Such a tendency might not be detected by the analysis of variance. If one assumes that under the conditions of our experiment there are equal chances that positive and negative potential differences will occur (an assumption that may be justified because the mean p.d. prior to any treatment of the animals deviates from zero by only +0.44 mv., and this deviation only just reaches significance [$P=0.05$]), the tendency of runs can be tested by the method developed by Wishart and Hirschfeld (20). These authors showed that if the occurrence of two events *a* and *b* has the same probability ($P=0.5$), the total number of both occurrences being $n$, then the number of sets of sequences of *a* and *b* (counting a single occurrence also as a run) has a binomial distribution with the mean $\bar{x}=\frac{n-1}{2}$ and a standard deviation $\sigma=\frac{\sqrt{n}}{2}$. The probability of the occurrence of the observed number of runs ($x$) in the animal was calculated by regarding (as an approximation) $\frac{\bar{x}-x}{\sigma}$ as a normal deviate, and using tables of the normal probability integral (Fisher and Yates, 8). The results of these calculations are given in column 10 of Tables IA and IB. However, in order not to prejudice our results through assuming equal chances for the occurrence of $+ve$ and $-ve$ p.d.'s, the test devised by Stevens (19) was also applied. By means of this test one can calculate from the *observed frequency* of positive and negative potential differences the probability of the occurrence of an observed number of sequences. The results of these calculations are shown in column 11, Tables IA and IB.

Finally it might be argued that if carcinogenesis has not any definite effect on the *level* (*i.e.*, positivity or negativity) of the potential difference, it might still result in an increase of *variation* (oscillation) of the potential difference values. Therefore in a third analysis the standard deviations of the measurements of potential differences were compared by the variance ratio test, calculated for the classifications 1 to 4 and 9 above, and the results are set out in Table IA and IB, columns 12 to 16 for both experimental and control animals. Thus in column 12 of Table I we have calculated the variance of the p.d.'s before the macroscopic detection of a papilloma and tested this against the variance of the remaining p.d.'s. Variance ratios were calculated similarly for the other classifications. In each case the hypothesis was tested that carcinogenesis increases the variance.

### C. Tests for All Animals Taken Together

Any correlation between the potential differences and different stages of tumor development will be made more obvious by pooling the corresponding results for all animals, and this has been carried out in the following ways:

(a) Average levels of p.d.'s were calculated by adding the p.d. measurements for all animals taken at the corresponding stages, and this was done for all the classifications set out above. The average levels were then compared by the t-test to see whether any change in the level of p.d. could be associated with any of the classifications. In order to control any possible bias of these means through painting on the right or left side of the animal, the average levels were calculated separately for the two sides in each classification. The results of these tests are given in Table II.

In a similar manner the average levels of p.d. were compared for the following classifications: (i) *o*, *p*, *m*, and controls *c*; results in Table III; (ii) 5 measurements immediately preceding and during the early stages of development of a papilloma, $P'$, and the rest of the measurements, $R$; Table IVA; (iii) 5 measurements immediately preceding and during the early stages of development of malignancy, $M'$, and the rest of the measurements, $R$; Table IVB.

(b) The classifications shown in columns 12 to 16 of Tables IA and IB and in Tables III and IV have also been investigated to determine whether they are in any way associated with a change in variance of

TABLE Ia: (EXPERIMENTAL MICE) RESULTS OF ANALYSIS OF VARIANCE AND VARIANCE RATIO TESTS

| A. INDIVIDUAL MICE Animal serial no. | Duration of experiment, days | No. of measurements | Tumor development | [1] | [2] | [3] | [4] | [5] | [6] | [7] | [8] | [9] | [10] | [11] | [12] | [13] | [14] | [15] | [16] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 150 | 45 | *p* | *n* | — | *w* | — | *n* | 0.05 | *w* | *w* | *w* | *n* | *n* | *n* | — | *n* | — | *n* |
| 2 | 150 | 58 | *p* | *w* | — | *w* | — | *n* | *n* | *w* | *w* | *n* | *n* | *n* | *n* | — | *n* | — | 0.05 |
| 3 | 133 | 53 | *m* | *w* | *w* | *n* | *w* | *n* | *w* | *w* | *w* | *w* | *n* | 0.01 | 0.05 | 0.05 | *n* | 0.05 | *n* |
| 4 | 129 | 43 | *m* | *w* | *w* | *w* | 0.05 | *w* | *n* | *w* | *n* | 0.05 | *n* | *n* | *n* | 0.05 | *n* | *n* | *n* |
| 5 | 50 | 23 | *o* | — | — | — | — | 0.05 | *n* | *n* | — | — | *n* | *n* | — | — | — | — | — |
| 6 | 70 | 22 | *p* | *w* | — | *w* | — | *w* | *w* | *n* | — | — | *n* | *n* | 0.05 | — | *n* | — | — |
| 7 | 56 | 21 | *o* | — | — | — | — | 0.05 | *n* | *n* | — | — | *n* | *n* | — | — | — | — | — |
| 8 | 70 | 22 | *o* | — | — | — | — | *w* | *w* | *w* | — | — | *n* | *n* | — | — | — | — | — |
| 9 | 73 | 23 | *p* | *w* | — | *w* | — | *w* | *n* | *w* | — | — | *n* | *n* | *n* | — | *n* | — | — |
| 10 | 143 | 43 | *m* | *w* | *w* | *n* | *n* | *n* | 0.01 | *w* | *w* | 0.05 | 0.01 | 0.02 | 0.05 | 0.05 | *n* | 0.05 | *n* |
| 11 | 140 | 42 | *p* | *w* | — | 0.05 | — | *n* | 0.001 | *n* | *n* | 0.05 | 0.02 | 0.005 | *n* | — | 0.05 | — | 0.01 |
| 12 | 136 | 41 | *m* | *w* | *n* | *w* | 0.01 | *w* | *n* | *w* | *w* | *w* | *n* | *n* | 0.05 | *n* | *n* | *n* | *n* |
| 13 | 136 | 41 | *p* | *w* | — | *w* | — | *w* | 0.001 | *n* | *w* | *w* | *n* | *n* | 0.05 | — | *n* | — | *n* |
| 14 | 136 | 41 | *m* | *w* | *w* | *n* | *n* | *w* | 0.05 | *w* | *n* | 0.001 | *n* | *n* | *n* | *n* | *n* | 0.01 | *n* |
| 15 | 133 | 40 | *p* | *n* | — | *n* | — | *n* | *n* | *n* | *w* | *w* | *n* | *n* | *n* | — | *n* | — | *n* |
| 16 | 130 | 48 | *m* | 0.05 | *n* | *w* | *w* | *n* | *n* | *w* | *w* | *w* | *n* | *n* | *n* | *n* | *n* | *n* | *n* |
| 17 | 150 | 56 | *p* | *w* | — | 0.01 | — | 0.05 | 0.001 | *w* | *w* | *w* | 0.001 | 0.02 | *n* | — | *n* | — | *n* |
| 18 | 80 | 25 | *p* | *w* | — | *w* | — | *w* | *w* | *w* | — | — | *n* | *n* | *n* | — | *n* | — | — |
| 19 | 80 | 25 | *p* | *n* | — | *n* | — | 0.05 | *n* | *n* | — | — | *n* | *n* | *n* | — | *n* | — | — |
| 20 | 70 | 22 | *p* | *w* | — | *w* | — | *w* | *w* | *w* | — | — | 0.05 | *n* | — | — | 0.05 | — | — |
| 21 | 150 | 45 | *m* | — | *w* | — | *w* | *n* | *n* | *w* | *w* | *w* | *n* | *n* | — | 0.01 | — | 0.05 | 0.05 |
| 22 | 56 | 21 | *p* | *w* | — | *n* | — | *n* | *n* | *w* | — | — | *n* | *n* | *n* | — | *n* | — | — |
| 23 | 56 | 21 | *m* | — | *n* | *n* | *n* | 0.01 | 0.05 | *n* | — | — | *n* | *n* | — | *n* | — | *n* | — |
| 24 | 150 | 45 | *m* | 0.05 | 0.05 | — | 0.001 | 0.05 | 0.05 | *w* | *w* | *w* | 0.015 | *n* | *n* | 0.01 | — | *n* | *n* |
| 25 | 49 | 19 | *o* | — | — | — | — | *n* | *w* | *w* | — | — | *n* | *n* | — | — | — | — | — |
| B. POOLED MICE | | 970 | — | 0.2 | 0.2 | 0.05 | 0.01 | 0.01 | 0.00001 | — | — | 0.2 | 0.001 | 0.00012 | — | — | — | — | — |

TABLE Ib: (CONTROL MICE) RESULTS OF ANALYSIS OF VARIANCE AND VARIANCE RATIO TESTS

| Animal serial no. | Duration of experiment, days | No. of measurements | Tumor development | [1] | [2] | [3] | [4] | [5] | [6] | [7] | [8] | [9] | [10] | [11] | [12] | [13] | [14] | [15] | [16] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A. INDIVIDUAL MICE** | | | | | | | | | | | | | | | | | | | |
| I | 150 | 45 | — | *w* | *w* | *w* | *n* | *n* | *n* | *w* | *w* | *w* | *n* | *n* | 0.01 | *n* | *n* | *n* | *n* |
| II | 150 | 45 | — | *w* | *n* | *w* | *w* | *n* | 0.05 | *w* | *w* | 0.05 | *n* | *n* | 0.001 | 0.01 | 0.05 | 0.05 | *n* |
| III | 150 | 44 | — | *n* | 0.05 | *w* | *n* | *n* | 0.05 | *w* | *w* | *w* | *n* | *n* | *n* | *n* | *n* | *n* | *n* |
| IV | 150 | 45 | — | *w* | *w* | 0.01 | 0.01 | *w* | *n* | *w* | *w* | *w* | *n* | *n* | *n* | *n* | *n* | *n* | *n* |
| V | 150 | 45 | — | *w* | 0.01 | *w* | *n* | *n* | *w* | *w* | *w* | 0.05 | *n* | *n* | 0.001 | 0.01 | *n* | *n* | *n* |
| VI | 150 | 45 | — | *w* | *n* | *w* | *w* | *n* | 0.05 | *w* | *w* | *n* | *n* | *n* | 0.05 | *n* | 0.05 | *n* | *n* |
| VII | 150 | 45 | — | *w* | *n* | *n* | *w* | *n* | *n* | *n* | *n* | *n* | *n* | *n* | 0.01 | *n* | *n* | *n* | *n* |
| VIII | 150 | 45 | — | *w* | *w* | *n* | *n* | *w* | 0.05 | *w* | *w* | *w* | *n* | *n* | *n* | *n* | *n* | *n* | *n* |
| IX | 77 | 24 | — | *n* | — | *w* | — | *n* | *w* | *w* | — | — | *n* | *n* | *n* | *n* | *n* | *n* | *n* |
| X | 150 | 45 | — | *w* | *w* | *w* | 0.05 | *w* | 0.05 | *w* | *w* | *w* | 0.04 | *n* | *n* | — | *n* | — | — |
| **B. POOLED MICE** | | 428 | — | *w* | 0.1 | *w* | 0.1 | 0.2 | 0.01 | — | — | 0.2 | 0.02 | 0.0107 | — | — | — | — | — |

p.d. This was done by pooling the variances found for individual animals at corresponding stages, and the results are shown in Table V. The suffix *e* or *c* added to headings *N*, *D*, *QP*, etc., indicates experimental or control group of animals respectively, and the group having the larger variance is given as numerator in each ratio heading, *e.g.*, *D* in *D*/*N*.

It should be noted that in these tables all the animals do not contribute to every classification, so that on adding the frequencies of the separate classifications in order to check the total frequencies, the same sum is not obtained in every check. For example, every experimental animal contributes to the early "clinical" classifications but only those that develop a papilloma contribute to the nonpapillomatous classification *X* in Table V.

(c) The results of the Wishart and Hirschfeld test were pooled by the method described by Fisher (7) and the pooled result is shown in the bottom lines of Tables IA and IB, column 10.

(d) The results of Stevens' tests were pooled by calculating the average of the normal deviates, their standard deviation, and the ratio $\frac{\text{average normal deviate}}{\text{standard deviation}}$, and the results are given in the bottom lines of column 11 Tables IA and IB.

The results of these tests are summarized in the following section and are discussed in greater detail in the appendix to this paper.

## VI. SUMMARY

Potential differences have been measured between positions on the surface of the tumor and neighboring normal tissue in the skin of MRC mice, the tumors being produced by painting with the carcinogen 9,10-dimethyl-1,2-benzanthracene in acetone. P.d.'s were recorded between a painted area and an unpainted area of skin on which a grounded electrode was placed. Unless otherwise stated the p.d.'s were therefore either positive or negative with respect to the unpainted skin as reference point. The changes in p.d. occurring during carcinogenesis in these animals have been compared with similar measurements in control mice painted with acetone.

When animals are examined individually a statistical analysis of the measurements shows only one significant result; *i.e.*, that at the "clinical" stages distinguished in this experiment in the course of carcinogenesis the p.d.'s tend to remain at a given level, although at certain stages there may be great diversity of p.d. about this level.

When the measurements for all animals are taken together in various groupings a statistical analysis shows that:

1. Painting with either acetone or with the carci-

TABLE II: MEAN POTENTIAL DIFFERENCES, THEIR STANDARD ERRORS, AND SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEANS IN "CLINICAL" GROUPS

A

| | | Animals painted left side | | | | | | Animals painted right side | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Clinical" group | *N* | *D* | *QP* | *P* | *QM* | *M* | *N* | *D* | *QP* | *P* | *QM* | *M* |
| Experimental mice | Mean ($\bar{x}$) | −0.17 | −0.16 | +0.51 | −0.77 | −0.36 | −0.05 | +0.33 | −3.10 | −4.07 | −0.27 | −0.74 | +1.89 |
| | Standard error (s. e.) | 0.325 | 0.428 | 0.750 | 0.409 | 1.095 | 0.684 | 0.411 | 0.883 | 1.060 | 0.689 | 1.363 | 0.696 |
| | Frequency | 94 | 136 | 53 | 187 | 26 | 61 | 66 | 78 | 52 | 60 | 23 | 43 |
| | Significance of differences between $\bar{x} - \bar{x}'$ | *n* | *n* | *n* | *n* | *n* | | 0.001 | *n* | 0.001 | *n* | 0.05 | |
| Control mice | Mean ($\bar{x}$) | −0.77 | −0.48 | −0.58 | −0.28 | −1.17 | +1.90 | −1.15 | −0.77 | −0.75 | −1.15 | −1.15 | +0.40 |
| | Standard error (s. e.) | 0.493 | 0.716 | 1.551 | 0.529 | 1.049 | 0.961 | 0.706 | 0.524 | 0.737 | 0.569 | 1.274 | 0.825 |
| | Frequency | 29 | 40 | 20 | 60 | 35 | 40 | 30 | 40 | 20 | 54 | 28 | 32 |
| | Significance of differences between $\bar{x} - \bar{x}'$ | *n* | *n* | *n* | *n* | 0.05 | | *n* | *n* | *n* | 0.05 | *n* | |
| | Significance of differences between $\bar{x} - \bar{x}'$ of corresponding groups of experimental and control mice | *n* | *n* | *n* | *n* | *n* | 0.05 | 0.02 | *n* | *n* | *n* | *n* | *n* |

B

| | | All animals, considered: painted/unpainted side | | | | | | All animals, considered: left/right side | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Clinical" groups | *N* | *D* | *QP* | *P* | *QM* | *M* | *N* | *D* | *QP* | *P* | *QM* | *M* |
| Experimental mice | Mean ($\bar{x}$) | +0.04 | −1.23 | −1.76 | −0.65 | −0.54 | +0.75 | −0.14 | +1.03 | +2.27 | −0.51 | +0.17 | −0.81 |
| | Standard error (s. e.) | 0.814 | 0.442 | 0.706 | 0.353 | 0.953 | 0.502 | 0.814 | 0.437 | 0.675 | 0.353 | 0.953 | 0.507 |
| | Frequency | 160 | 214 | 105 | 247 | 49 | 104 | 160 | 214 | 105 | 247 | 49 | 104 |
| | Significance of differences between $\bar{x} - \bar{x}'$ | 0.05 | *n* | *n* | *n* | *n* | | 0.05 | *n* | 0.001 | *n* | *n* | |
| Control mice | Mean ($\bar{x}$) | −0.96 | −0.63 | −0.67 | −0.69 | −0.12 | +1.23 | +0.21 | +0.15 | +0.08 | +0.40 | −1.16 | +0.91 |
| | Standard error (s. e.) | 0.449 | 0.454 | 0.885 | 0.453 | 0.831 | 0.672 | 0.459 | 0.454 | 0.887 | 0.450 | 0.826 | 0.669 |
| | Frequency | 59 | 80 | 40 | 114 | 63 | 72 | 59 | 80 | 40 | 114 | 63 | 72 |
| | Significance of differences between $\bar{x} - \bar{x}'$ | *n* | *n* | *n* | *n* | *n* | | *n* | *n* | *n* | *n* | 0.05 | |
| | Significance of differences between $\bar{x} - \bar{x}'$ of corresponding groups of experimental and control mice | 0.05 | *n* | *n* | *n* | *n* | *n* | *n* | *n* | *n* | *n* | *n* | 0.05 |

nogen in acetone has no influence on the p.d.'s measured.

2. In all the animals prior to any painting and in animals painted with acetone alone (control animals), the mean p.d.'s reveal an electrical asymmetry, the left side of the animal being positive. This asymmetry increases with age in the control animals, with the exception of a disturbance at the "clinical" stage *QM*. The variance, and thus the standard deviation of the p.d.'s, increases similarly with age.

3. In animals painted with the carcinogen in acetone the results obtained depend upon the classifications chosen for investigation. Association with the level or variance of p.d., or with the sign of the mean p.d., or with all these was found as follows: (a) the mean p.d. for all the experimental animals taken together, as well as the mean p.d.'s for the experimental animals arranged in groups according to the histological stage reached at death, differs significantly from the mean p.d. in the control animals; (b) there is a greater variance and standard deviation of the p.d.'s (*i.e.*, a greater diversity) in the dry and hypertrophic stages of the skin preceding the appearance of a papilloma than in the normal (unpainted), papillomatous, or malignant stages; (c) this variance is greatest during the hypertrophic stage; (d) the variance during the malignant stage is less than during the rest of the experiment; (e) there is no correlation between the *sign* of the mean p.d. and the "clinical" stages in carcinogenesis distinguished in our experiment; (f) in the period immediately preceding and during the early stages of development of a papilloma there is some evidence that the mean p.d. is more negative than during the rest of the experiment, and there is a greater variance and a change in the level of the p.d. during this period. The more negative p.d. during this period may indicate an effect different from wounding of the skin, for a wounded area shows a positive p.d. with respect to an uninjured area. The importance of this period in tumor development has been suggested by other workers. (g) In the period immediately preceding and during the early stages of malignancy there is no change in the mean p.d. nor in the variance, but there is a definite change in the level of the p.d.; (h) the electrical asymmetry is not as consistent as in the control animals and thus it appears to be disturbed by tumor development. This inconsistency seems to be caused mainly by the contribution of those animals painted on the right side.

4. In both control and experimental sets of mice the values of p.d.'s measured close together in time are not quite independent, and therefore in studying such a problem it is statistically preferable to measure p.d.'s at reasonably long intervals on a large number of animals rather than to take frequent measurements on few animals.

Further statistical tests carried out showed no significant results.

The significance of the changes in p.d. observed in this experiment, and of the p.d.'s themselves, is obscure.

TABLE III: MEAN POTENTIAL DIFFERENCES, THEIR STANDARD ERRORS, AND SIGNIFICANCE OF DIFFERENCES BETWEEN THE MEANS IN GROUPS ARRANGED ACCORDING TO HISTOLOGICAL STAGE REACHED AT DEATH

| | Control mice | Experimental mice | | | |
|---|---|---|---|---|---|
| | *c* | *o* | *p* | *m* | Total |
| Mean ($\bar{x}$) | +0.15 | +1.39 | −0.52 | −0.72 | −0.51 |
| Standard error (s. e.) | 0.230 | 0.645 | 0.221 | 0.295 | 0.175 |
| Frequency | 428 | 85 | 505 | 380 | 970 |
| Significance of differences between $\bar{x}$ in control and $\bar{x}$'s in experimental mice | | 0.01 | 0.05 | 0.02 | 0.05 |

As almost all the significant changes were observed in the pooled experimental measurements and not in individual animals, this technic cannot be used at present for the early diagnosis of tumors.

## VII. APPENDIX. DISCUSSION OF RESULTS

We propose to discuss our results in the following order: (a) p.d.'s of the skin prior to any treatment, (b) p.d.'s in control animals, (c) p.d.'s in experimental animals.

### (a) P.D.'s PRIOR TO ANY TREATMENT

As there is no obvious anatomical difference between the skin of the left and right sides of the back of the mouse one would expect that on the average the p.d. between the skin on the two sides of the back would be zero. Prior to any painting, however, the mean p.d. in our pooled control and experimental animals is +0.444 mv. (left side positive with respect to the right side). This small deviation from zero is just statistically significant and may indicate an electrical asymmetry in the animals, but we cannot offer any explanation of this. We show in the following paragraph that 5 consecutive measurements within a fortnight are not quite independent, and this should

TABLE IV: MEAN POTENTIAL DIFFERENCES, THEIR STANDARD ERRORS, AND SIGNIFICANCE OF DIFFERENCES BETWEEN MEANS IN:—

A. Prepapillomatous group ($P'$) and the rest ($R$)

| | Control mice | | | | | | Experimental mice | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Left | | Right | | Total | | Left | | Right | | Total | |
| | $P'$ | $R$ | $P'$ | $R$ | $P'$ | $R$ | $P'$ | $R$ | $P'$ | $R$ | $P'$ | $R$ |
| Mean ($\bar{x}$) | −0.18 | −0.12 | −1.11 | −0.40 | −0.65 | −0.25 | −1.21 | −0.45 | −4.21 | +0.02 | −2.21 | −0.32 |
| Standard error (s. e.) | 0.652 | 0.362 | 0.866 | 0.346 | 0.546 | 0.253 | 0.612 | 0.239 | 1.081 | 0.452 | 0.657 | 0.209 |
| Frequency | 25 | 199 | 25 | 176 | 50 | 375 | 60 | 432 | 30 | 167 | 90 | 599 |
| Significance of differences between $\bar{x} - \bar{x}'$ | $n$ | | $n$ | | $n$ | | $n$ | | 0.001 | | 0.001 | |

| B. Premalignant group ($M'$) and the rest ($R$) | $M'$ | $R$ | $M'$ | $R$ | $M'$ | $R$ | $M'$ | $R$ | $M'$ | $R$ | $M'$ | $R$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean ($\bar{x}$) | −0.04 | −0.09 | +0.35 | −0.52 | +0.13 | −0.28 | −3.03 | −0.64 | +1.51 | −1.08 | −0.81 | −0.82 |
| Standard error (s. e.) | 0.958 | 0.352 | 1.485 | 0.355 | 0.828 | 0.252 | 1.018 | 0.357 | 1.202 | 0.573 | 0.817 | 0.326 |
| Frequency | 25 | 199 | 20 | 160 | 45 | 359 | 25 | 196 | 24 | 135 | 49 | 331 |
| Significance of differences between $\bar{x} - \bar{x}'$ | $n$ | | $n$ | | $n$ | | 0.05 | | $n$ | | $n$ | |

TABLE V: SIGNIFICANCE OF VARIANCE RATIOS

| | | [1] | [2] | [3] | [4] | [5] | [6] | [7] | [8] | [9] |
|---|---|---|---|---|---|---|---|---|---|---|
| (a) Experimental (Total) | Classifications compared | $D/N$ | $QP/D$ | $QP/P$ | $QM/P$ | $QM/M$ | $P'/R$ | $M'/R$ | $R'/X$ | $Y/M$ |
| | Frequencies | 214/160 | 105/214 | 105/247 | 49/247 | 49/105 | 84/597 | 45/335 | 352/317 | 276/105 |
| | Significance of ratio | 0.001 | $n$ | 0.001 | $n$ | 0.05 | 0.05 | $n$ | 0.05 | 0.05 |
| (b) Control (Total) | Classifications compared | $D/N$ | $D/QP$ | $P/QP$ | $QM/P$ | $QM/M$ | $R/P'$ | $R/M'$ | $R'/X$ | $M/Y$ |
| | Frequencies | 80/59 | 80/40 | 114/40 | 63/114 | 63/72 | 378/50 | 359/45 | 249/179 | 72/332 |
| | Significance of ratio | $n$ | $n$ | 0.01 | 0.05 | $n$ | 0.05 | $n$ | 0.001 | 0.01 |
| (c) Experimental and control (Total) | Classifications compared | $Nc/Ne$ | $De/Dc$ | $QPe/QPc$ | $Pe/Pc$ | $QMe/QMc$ | $Mc/Me$ | $P'e/P'c$ | $Xe/Xc$ | $R'e/R'c$ | $Ye/Yc$ | $M'e/M'c$ |
| | Frequencies | 59/160 | 214/80 | 105/40 | 247/114 | 49/63 | 72/105 | 84/48 | 317/179 | 352/249 | 276/332 | 45/45 |
| | Significance of ratio | $n$ | 0.001 | 0.001 | 0.05 | $n$ | $n$ | 0.001 | 0.001 | $n$ | 0.001 | $n$ |
| (Left side) | Classifications compared | $Ne/Nc$ | $De/Dc$ | $QPc/QPe$ | $Pe/Pc$ | $QMc/QMe$ | $Mc/Me$ | $P'e/P'c$ | $Xc/Xe$ | $R'c/R'e$ | $Yc/Ye$ | |
| | Frequencies | 94/29 | 136/40 | 20/53 | 187/60 | 35/26 | 40/61 | 60/25 | 89/217 | 135/274 | 184/160 | |
| | Significance of ratio | $n$ | $n$ | 0.05 | $n$ | $n$ | $n$ | 0.001 | $n$ | $n$ | $n$ | |
| (Right side) | Classifications compared | $Nc/Ne$ | $De/Dc$ | $QPe/QPc$ | $Pe/Pc$ | $QMc/QMe$ | $Mc/Me$ | $P'e/P'c$ | $Xe/Xc$ | $R'e/R'c$ | $Ye/Yc$ | |
| | Frequencies | 30/69 | 78/40 | 52/20 | 60/54 | 28/23 | 32/43 | 30/25 | 196/90 | 126/114 | 116/148 | |
| | Significance of ratio | $n$ | 0.001 | 0.001 | 0.01 | $n$ | $n$ | 0.05 | 0.001 | $n$ | 0.01 | |

$X = N + D + QP$ $Y = N + P + QM$ $R' = P + QM + M$

be remembered when considering the significance of the results above.

### (b) P.D.'s in Control Animals

The mean p.d. throughout the experiment for all control animals is found to be +0.152 mv., which remains unchanged when the correction to a zero of +0.444 mv. is applied, since there is an equal number of animals painted on the right and left sides, and their contributions to the correction therefore cancel (Table III). A detailed discussion of the mean p.d.'s at different stages in the control animals is given in section (c).

From Table Ib, columns 7, 8, and 9 it is clear that painting with acetone has no influence on the p.d. However, the results for the pooled animals in Table Ib, columns 10 and 11, bottom line, show that there is a tendency for p.d.'s of the same sign to occur in longer sequences (consequently the runs occur less frequently) than would be expected by chance. This is confirmed by the result set forth in the bottom line of column 6 in Table Ib, which shows that there is a greater similarity between the p.d.'s within a group of 5 consecutive measurements (over a period of 14 days) than between successive groups.[1]

On comparing the variances in the pooled control animals at successive "clinical" stages it is observed that the variance, and thus the standard deviation, increases with age (Fig. 1). Burr (3) states that in both control and experimental Strong CBA mice, in which tumors were induced by subcutaneous injection of methylcholanthrene dissolved in sesame oil, a *decrease in variability* of p.d.'s was observed during the course of the experiment. It is clear that this result is not confirmed for our animals.

### (c) P.D.'s in Experimental Animals

*Pooled mean p.d.'s.*—The mean p.d. throughout the experiment for the pooled experimental animals is −0.36 mv., which corrected to our zero of +0.444 mv. gives an $\bar{x}$ of −0.51 mv. (Table III). From a consideration of the standard error it is seen that this value is significantly different from the mean p.d. prior to any painting, and this is also true for the mean p.d.'s of the classifications *o*, *p*, and *m*, but is not true for the controls. (From the method of calculation it is obvious that the corrected $\bar{x}$'s in Table III are already the differences between the mean p.d. prior to any painting and the $\bar{x}$'s of the classification concerned.)

The differences between the mean p.d. in the control group *c* and those in the classifications *o*, *p*,

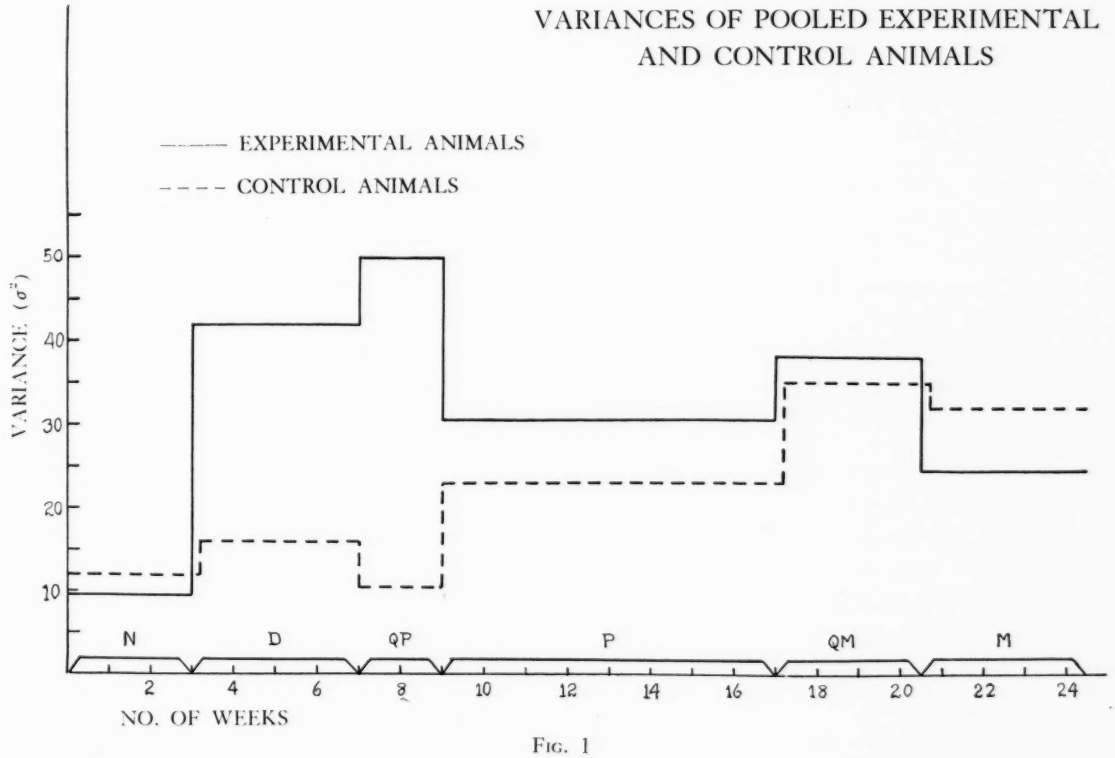


Fig. 1

[1] In applying the statistical tests above, the individual p.d. measurements are regarded as independent observations. Our results indicate that 5 consecutive measurements within a fortnight are not quite independent. Thus, in studying such a problem, it is statistically preferable to measure p.d.'s at reasonably long intervals on a large number of animals rather than to take frequent measurements on few animals.

*m*, and Total in the experimental group are also significant (Table III, bottom line).

*Effect of painting with carcinogen, and tendency for long sequences.*—It is clear that painting with 9,10-dimethyl-1,2-benzanthracene in acetone does not influence the p.d.'s in the experimental animals, as was found in the case of control animals painted with acetone alone (Table IA and IB, columns 7, 8, and 9). In these animals also there is a tendency for p.d.'s of the same sign to occur in longer sequences than would be expected by chance (Table IA, columns 10,11, and 6, bottom line), (but the probability of this occurrence is much smaller than in the control animals). However, testing shows that the difference between these results in the two sets of animals is not statistically significant.

*P.d.'s at different "clinical" stages during tumor development.*—A considerable increase in the variance of the p.d.'s in the pooled experimental animals occurs immediately after the skin becomes dry (*D*); (Fig. 1, and Table V, column 1, section [a]), *i.e.*, as the first result of the action of the carcinogen on the skin. This rise is statistically significant when compared with the corresponding stage in the pooled control animals.[2] This increase of the variance persists into the hypertrophic (doubtful papilloma, *QP*) stage. It then decreases as the papilloma appears but still in this stage *P* is just significantly greater than the corresponding stage in the controls. After this there is no significant difference between the variances in the two sets of animals. These results show, therefore, that in the stages *D* and *QP* preceding the appearance of a papilloma there is a great diversity of p.d.'s in the pooled experimental animals. This is followed by a greater uniformity at the papillomatous and malignant stages.

It is seen that so far significant results have not been obtained with individual animals, but only when the animals have been taken together in groups and their measurements pooled. This is not true when an analysis of variance is applied to the measurements in individual animals divided according to "clinical" groups. In this case 6 out of 25 carcinogen-painted animals show statistically significant changes in the p.d., although no such changes are observed in the controls (Tables IA and IB, column 5). This test indicates that at a definite "clinical" stage the p.d.'s tend to stay at the same level, although there may be considerable diversity of the p.d.'s about this level at certain stages; *e.g.*, in the *QP* stage. The former result is again still more emphasized on pooling the measurements (Table IA, column 5, bottom line). However, although there is this relationship between the *level* of the p.d.'s and definite "clinical" stages, we find no correlation between these stages and the *sign* of the p.d. One might expect that for a given change in the condition of the skin one would always observe, on the average, a change of the p.d. in the same direction, either positive or negative. In Table IIA, however, we see that the clinical groups *N*, *QP*, and *M* do not show this result, but that the sign of the potential differs according to the side on which the animal is painted. There is also no systematic significant difference between the mean p.d.'s of successive "clinical" groups, nor between those of corresponding "clinical" groups in experimental and control animals when the measurements for animals painted on the left side, and for those painted on the right side, are kept separate (see Table IIA).

When the p.d.'s at the different "clinical" stages are pooled for all the experimental animals, and are considered as usual, painted side against unpainted side of the animal (referred to in Tables as painted/unpainted side), it is seen that there is a significant difference between the mean p.d.'s in the *N* and *D* stages (and similarly between the *N* and *QP* stages) and the sign of the p.d. changes from positive to negative at the *D* stage and remains negative until malignancy *M* (see Table IIB and Fig. 2). Calculating similarly the measurements for the pooled control animals it is seen that there is no significant difference between the mean p.d.'s in successive "clinical" stages, although the sign of the p.d.'s is similar to that in the experimental animals at the different stages. Apart from the stage *N* the differences between the mean p.d.'s of corresponding groups of experimental and control animals (Table IIB) are not significant.

When the p.d.'s of all the experimental animals are pooled as above, but the signs are considered: left side against right side of the animal (*i.e.*, the signs of measurements on animals painted on the right side are reversed), the differences between the means at the *N* and *D* stages and the *N* and *QP* stages are again significant, and in addition there is a significant difference between the means at the *QP* and *P* stages (Table IIB and Fig. 3). Clearly the greater contribution to these results is made by those animals painted on the right side (Table IIA), again suggestive of an electrical asymmetry in the animals. Analyzing the curve for the mean p.d.'s of the control animals, considered: left/right side (Fig. 3), it is seen that between the stages *N* and *P* there is indeed an electrical asymmetry present in these animals, which is identical in sign and magnitude with that observed previous

[2] Where the experimental group is compared with the control group the two periods that are being compared do not correspond strictly to the same time period. No bias is introduced through this, for we have determined the various "clinical" stages for the control group in such a way that they correspond to the average time interval of the comparable "clinical" stage for the experimental animals.

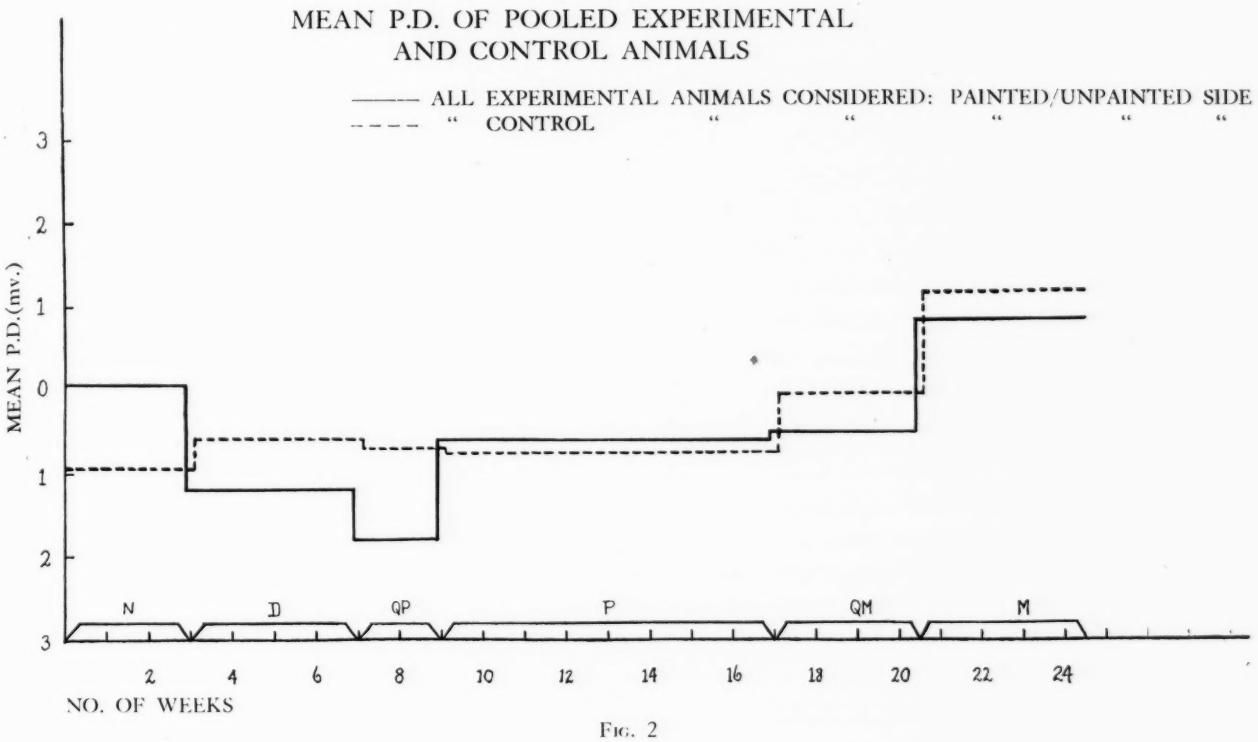
MEAN P.D. OF POOLED EXPERIMENTAL AND CONTROL ANIMALS
——— ALL EXPERIMENTAL ANIMALS CONSIDERED: PAINTED/UNPAINTED SIDE
- - - - " CONTROL " " " " "
MEAN P.D.(mv.)
3
2
1
0
1
2
3
N
D
QP
P
QM
M
2
4
6
8
10
12
14
16
18
20
22
24
NO. OF WEEKS

FIG. 2

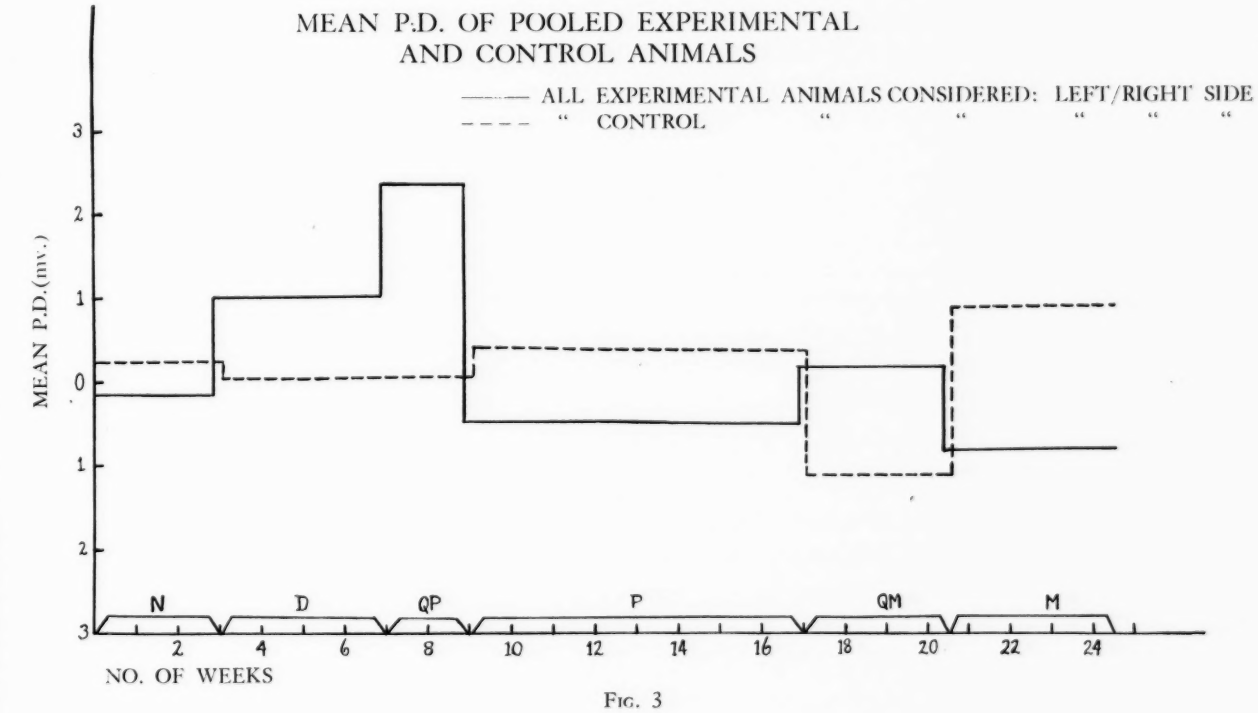
MEAN P.D. OF POOLED EXPERIMENTAL AND CONTROL ANIMALS
——— ALL EXPERIMENTAL ANIMALS CONSIDERED: LEFT/RIGHT SIDE
- - - - " CONTROL " " " " "
MEAN P.D.(mv.)
3
2
1
0
1
2
3
N
D
QP
P
QM
M
2
4
6
8
10
12
14
16
18
20
22
24
NO. OF WEEKS

FIG. 3

to any painting (+0.444 mv.). From the curves for the experimental animals and the results shown in Table IIA it is clear that there is no consistent asymmetry due to tumor development and in the *D* and *QP* stages the right side becomes much more negative than the left side. At present we cannot explain this result. A positive mean p.d. that increases with age is noticed in the control animals although this is disturbed at the *QM* stage, where it becomes negative and significantly different from *M*. This is not true for the experimental animals, for in the *M* stage their mean p.d. is positive and significantly different from that in the control animals.

*P.d.'s before the appearance of (a) a papilloma and (b) malignancy, compared with the rest.*—It should be observed that the "clinical" stages distinguished in our experiment are not necessarily significant stages in tumor development. Furthermore, nearly always during the development of a tumor there is at the later stages, *e. g.,* after the appearance of a papilloma, a superficial injury, hemorrhage, or necrosis, so that we know we are not always measuring actively growing, healthy tumor tissue. It has been shown that wounding the skin in rats causes an increase in p.d. between the wound and the normal skin, the wound being positive to the uninjured region (Burrows, Iball, and Roe, (5), and similar effects may complicate our results. Thus any relationship between a "clinical" stage and changes in p.d. may be irrelevant for the purpose of correlating tumor development with changes in p.d. Columns 1, 2, 12, and 13 in Tables IA and IB, and columns 8 and 9 in Table V, show the results of tests devised to probe for any such direct relationship.

There is no statistically significant difference between the variances of the p.d.'s preceding the appearance of a papilloma and the rest (column 1) nor between those preceding malignancy and the rest (column 2) in either control or experimental animals, and thus there is no tendency for the p.d.'s to remain at the same level during any of these periods. In both control and experimental animals the greater variance occurs in the second half of the experiment (*i.e.,* after the appearance of the papilloma in the experimental animals, as is seen from the variance ratios in Table V, column 8). It should be noted also that the variance ratio: experimental/control animals in the former half of the experiment is significant. The experimental animals show the greater variance, and it is clear that those painted on the right side make the greater contribution to this result (column 8).

It has been pointed out already that the variance increases steadily with age in the control animals and thus reaches a maximum in the stage *M*. In the experimental animals, however, this is reversed and the variance is significantly smaller than the rest in the stage *M* (column 9, Table V). This confirms the result noted in our discussion of p.d.'s at different "clinical" stages during tumor development.

The mean p.d.'s at the stages before and after the appearance of a papilloma or malignancy have also been calculated, together with the significance of their differences. Apart from a significant difference between the mean p.d.'s in the malignant stage and the rest in both control and experimental animals, the results of these calculations confirm those previously obtained and are therefore not tabulated.

*P.d.'s during the stages immediately preceding the development of (i) a papilloma, P′ and (ii) a malignant tumor, M′.*—Tests similar to those discussed above were applied to the stages *P′* and *M′* in experimental and control animals, in order to compare our results with those of Burr and his associates (3, 3*), which are discussed later. It is clear from columns 3 and 4 in Table IA that there is a significant difference between the variances of the 5 measurements immediately preceding and during the early stages of (a) a papilloma and (b) malignancy when these are tested against the rest.

(i) *Prepapillomatous stage. Level, variance, and sign of p.d.*—The results in column 3, bottom line, Table IA, are statistically suggestive, and thus there is an indication that during the early stages of the appearance of a papilloma some change in the level of the p.d. occurs. By means of the variance ratios (Table V) the p.d.'s during this period were investigated further and they are indeed statistically different from the rest. In the control animals the variance of the 5 measurements comprising this stage is significantly smaller than the rest of the measurements; *i.e.*, the p.d.'s vary less than during the rest of the experiment. In the pooled experimental animals this is reversed, however, for these 5 measurements show a greater variance than the rest (Table V, column 6, |a| and |b|). When the variance ratio, experimental/control animals, is determined for this period this result is confirmed (Table V, column 7, |c|), for such a ratio would occur by chance only once in a thousand times. The same is true when the animals are divided according to the side painted (Table V, column 7, |c|).

The mean p.d. in the pooled experimental animals for the period just preceding and during the early stages of development of a papilloma is significantly more negative than during the rest of the experiment, when the measurements are considered: painted/unpainted side (Table IVA). When the measurements are divided according to the side painted, however, this mean p.d. is significantly different only in those animals painted on the right side, again suggestive

of an electrical asymmetry. The control animals do not show any significant differences between the means.

(ii) *Premalignant stage. Level, variance, and sign of p.d.'s.*—Similar calculations were carried out for the premalignant stage. It is seen from column 4, Table IA, that there is a significant change in the *level* of the p.d. during this stage, but there is no increase in the *variance* (Table V, column 7, |a|, and 9, |c|). No significant difference is observed between the mean p.d.'s of the premalignant stage and the rest, when the measurements are pooled and considered: painted/unpainted side (Table IVB). When the measurements are divided according to the side painted, those animals painted on the left side show a difference between the means that is just significant.

### Comparison with the Results of Other Workers

Burr, Smith, and Strong (3) followed the bioelectric potentials in Strong strain A mice having spontaneous adenocarcinoma of the mammary gland, and stated that "in those mice in which cancer appeared before the 260th day, marked rises amounting to several thousands of microvolts were encountered in the readings across the chest. . . . The voltage rise across the chest appeared in some instances from ten days to two weeks before the tumor was detected by palpation. . . . The data suggest that as the new growth proceeds the chest potentials go up until they reach a peak not long after the tumor becomes palpable. . . . So long as the tumors were in the phase of rapid growth they were usually negative." In our experiment, papillomas were detected between the 149th and 176th day, and in different animals these were judged to be malignant at various times before the 227th day. It is difficult to compare our results with those of Burr, for it would not be possible to distinguish our different "clinical" stages in developing mammary carcinoma, and we do not know which "clinical" stage corresponds to Burr's palpable tumor. In addition the strain of mice used and the experimental conditions were different in the two experiments.[3]

We can state from our results that in the period preceding and during the early stages of a papilloma (*i.e.*, papilloma as defined in our experiment) the electrical characteristics are different from those during the rest of the experiment, and there is some indication that the p.d.'s are more negative at this stage. It is interesting to observe that Leitch (11), reporting on the effect of cessation of the irritant on the development of experimental tar cancer, states that "the bias towards malignancy is given to the cells probably during the pre-neoplastic stage." Prior to this, Bang (1) had distinguished a "latent period" between the time when painting had rendered the cells biologically malignant and the appearance of invasive growth, painting meanwhile having been discontinued. Kennaway and Kennaway (10) also remark on an observation by Dean (6), who examined the connection between the occurrence of cancer of the penis and the age of circumcision. It is interesting to note the difference between (a) the change in p.d. produced on wounding the skin, when the injured area shows a positive p.d. with respect to uninjured skin, and (b) this tendency for a p.d. more negative than during the rest of the experiment to occur in the period preceding and during the early stages of a papilloma. This may indicate a change different from that associated with wounding of the skin. These observations draw attention to the importance for tumor development of the stages preceding the appearance of a papilloma, and also of their special electrical characteristics.

The period immediately preceding and during the early stages of malignancy also shows electrical characteristics different from those during the rest of the experiment and similar to those during the prepapillomatous stage. It must be remembered in this connection that the premalignant stage is much less clearly defined in our experiment than the prepapillomatous stage, since the macroscopic detection of malignancy is liable to greater error than the detection of a papilloma.

As almost all the significant changes are noticed in the pooled experimental results and not in individual animals, this technic cannot be used at present for the early diagnosis of tumors. The significance of the changes in p.d. and of the p.d.'s themselves is obscure.

We should like to express our indebtedness to Dr. H. O. Hartley, who has given invaluable advice on the statistical analysis of our results, and to Professor E. L. Kennaway for his interest and help. The investigation has been supported by generous grants, for which we express our thanks, from the British Empire Cancer Campaign. The International Cancer Research Foundation, The Anna Fuller Fund, and The Jane Coffin Childs Memorial Fund. One of us (Bruno Schober) wishes to thank the Department of Education of the Czechoslovak Ministry of the Interior for a full-time grant.

[3] In a later paper, however, Burr, Smith, and Strong (3*) reported on the bioelectric potentials in CBA mice in which tumors were induced by the external application of benzpyrene. In this experiment inflammation caused by painting the skin of the right axilla with benzene or with a solution of benzpyrene in benzene seemed to be associated with an increasingly negative mean p.d. at the right axilla with respect to the left. This negative mean p.d. reached a maximum in the papilloma stage in those animals treated with benzpyrene solution and decreased with the onset of malignancy. No statistical analysis is given of the differences between the mean p.d.'s at different stages or in animals undergoing different treatment.

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# Statistical Analysis of 2,407 Admissions to the Tumor Clinic of Veterans Hospital, Hines, Illinois, during 1943*

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The natural history of cancer in man has been studied by several methods, such as (a) review of the clinical histories of patients with specific types of cancer, (b) analysis of the mortality statistics in a country, and (c) survey of the incidence of cancer in a given locality.

A fourth method, comprehensive analysis of the histories of all patients with cancer in a large tumor clinic, was employed by Pack and LeFevre (3) for 19,129 patients at Memorial Hospital during 1917 to 1928 inclusive; by Daland and his staff (8) for 1,444 patients at Pondville Hospital during 1927 to 1929; and by Schrek (4) for 2,501 patients at Hines Veterans Hospital during 1941. Although this method is used infrequently because of the difficulties in analyzing and publishing a large mass of statistical data, it is useful in portraying the status of the cancer problem as observed in a large tumor clinic.

*Admissions.*—During 1943 the total number of admissions for neoplasia to the tumor clinic of Hines Veterans Hospital was 2,407 (Table I). This number included 1,623 primary admissions (first admissions in 1943), 581 readmissions (patients admitted prior to 1943 but readmitted in 1943), and 203 duplicate admissions (second and third admissions of the same patient in 1943). Only 32 women were treated for cancer at Hines Veterans Hospital during 1943.

TABLE I: NUMBER OF PATIENTS ADMITTED AND TOTAL NUMBER OF ADMISSIONS TO TUMOR CLINIC OF HINES VETERANS HOSPITAL DURING 1943; RACE AND SEX OF PATIENTS

| | Both sexes | | | | Male | | | | Female |
|---|---|---|---|---|---|---|---|---|---|
| | All races | White | Colored | Other races | All races | White | Colored | Other races | White |
| Primary admissions (patients admitted for the first time in 1943) | | | | | | | | | |
| Cancer | 1,462 | 1,346 | 114 | 2 | 1,445 | 1,329 | 114 | 2 | 17 |
| Benign tumor | 161 | 146 | 15 | 0 | 157 | 142 | 15 | 0 | 4 |
| Total primary admissions | 1,623 | 1,492 | 129 | 2 | 1,602 | 1,471 | 129 | 2 | 21 |
| Patients admitted or readmitted during 1943 | | | | | | | | | |
| Cancer | 2,021 | 1,879 | 140 | 2 | 1,993 | 1,851 | 140 | 2 | 28 |
| Benign tumor | 183 | 166 | 17 | 0 | 179 | 162 | 17 | 0 | 4 |
| Total patients | 2,204 | 2,045 | 157 | 2 | 2,172 | 2,013 | 157 | 2 | 32 |
| All admissions (including multiple admissions of same patient) | | | | | | | | | |
| Cancer | 2,222 | | | | 2,186 | | | | 36 |
| Benign tumor | 185 | | | | 181 | | | | 4 |
| Total admissions | 2,407 | | | | 2,367 | | | | 40 |

Of the 1,623 primary admissions, 161 patients had benign lesions. Some cases of benign tumors observed in the hospital are not included in this report, as the patients were treated for other diseases and were not referred to the tumor clinic for diagnosis or treatment of the growth.

The most common types of cancer seen in this clinic (Table II) were carcinoma of the exposed skin (306 cases, or 13.9 per cent of the 2,204 patients) and carcinoma of the lung (158 cases, or 7.2 per cent). There were also large groups of patients with carcinoma of the mouth and tongue (130 cases, or 5.9 per cent), of the larynx (111 cases, or 5.0 per cent),

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** With the assistance and advice of Dr. Max Cutler, Consultant, Tumor Clinic.

of the lip (111 cases, or 5.0 per cent), of the rectum and rectosigmoid (101 cases, or 4.6 per cent), and of the bladder (98 cases, or 4.4 per cent). Table II does not show the tumors that occurred in small groups of patients.

*Geographic distribution.*—Of the 1,623 primary admissions, only 304 patients (18.7 per cent, Table III) resided in the county in which Hines Veterans Hospital is situated, *i.e.,* Cook County, including the City of Chicago. An additional 158 persons (9.7 per cent) resided in the other counties of Illinois. Other states that contributed a large number of patients were Texas (140 cases, or 8.6 per cent), Ohio (109 cases, or 6.7 per cent), Indiana (109 cases, or 6.7 per cent), Michigan (96 cases, or 5.9 per cent), and Missouri (91 cases, or 5.6 per cent).

A large percentage of the patients (94.9 per cent) came from the Central states. Approximately half (51.2 per cent) were from the East North Central states, and 19.4 per cent from the West North Central. The Southern states contributed 25.3 per cent of the primary admissions (Table II).

Further analysis of the geographic distribution (Table III) showed that 22.0 per cent of the patients from Cook County and 16.5 per cent from the rest of Illinois had benign tumors, as compared with 5.0 per cent from the West South Central states and 9.92 per cent for all states. Evidently a relatively large number of patients with benign lesions came from the county and state in which the hospital is located, and relatively few from distant states. This is what one would expect *a priori.*

Approximately one-fourth of all the patients (25.69 per cent) resided in rural sections (Table III). Those from Southern states resided in rural districts in 40.0 per cent of the cases, but only 15.9 per cent from the East North Central states were rural.

Table II shows the relationship between the type of cancer and the geographic distribution of the patients. Of the 1,462 patients with cancer, 26.53 per cent were from Southern states. In contrast, 50.0 per cent of the patients with carcinoma of the exposed skin, and only 17 and 12 per cent of those with tumors of the stomach and the testis respectively, were from the South. These findings would suggest a high incidence of cancer of the exposed skin, and a low incidence of cancer of the stomach and testis, in the Southern states.

It is seen in Table II that 26.40 per cent of the 1,462 patients with cancer resided in rural areas. In contrast, a higher percentage (32.8 and 44.3 per cent) of the patients with cancer of the exposed skin and of the lip were from rural sections. The table also shows that rural sections supplied only 7 per

TABLE II: NUMBER OF PATIENTS WITH VARIOUS TYPES OF CANCER; GEOGRAPHIC AND RACIAL DISTRIBUTION AND AVERAGE AGE OF PATIENTS (PRIMARY ADMISSIONS)

| | Number of patients (primary admissions and readmissions | Primary admissions | | | | |
|---|---|---|---|---|---|---|
| | | Number of patients | Percentage of patients | | | Average age, years |
| | | | From Southern states | From rural sections | Colored | |
| All tumors | 2,204 | 1,623 | 25.26 | 25.69 | 7.94 | 50.14 |
| All cancer | 2,021 | 1,462 | 26.53 | 26.40 | 7.79 | 50.42 |
| All benign tumors | 183 | 161 | 12.42 * | 19.25 ** | 9.31 | 47.63 * |
| Carcinoma of: | | | | | | |
| Exposed skin | 306 | 180 | 50.0 * | 32.8 ** | 0.6 * | 53.08 * |
| Lip | 111 | 79 | 30 | 44 * | 0 * | 53.63 * |
| Mouth and tongue | 130 | 84 | 32 | 27 | 8 | 54.64 * |
| Larynx | 111 | 61 | 18 | 16 | 2 ** | 54.06 * |
| Lung | 158 | 145 | 26 | 23 | 9 | 51.89 |
| Esophagus | 43 | 42 | 19 | 19 | 31 * | 55.00 * |
| Stomach | 90 | 81 | 17 * | 21 | 6 | 52.31 |
| Rectum and rectosigmoid | 101 | 76 | 21 | 24 | 5 | 52.57 |
| Bladder | 98 | 71 | 23 | 34 | 10 | 53.93 * |
| Prostate | 52 | 37 | 16 | 22 | 16 | 58.58 * |
| Teratoma of testis | 51 | 41 | 12 ** | 39 | 0 | 32.90 * |
| Hodgkin's disease | 69 | 47 | 28 | 26 | 9 | 40.53 * |
| Leukemia | 93 | 52 | 19 | 31 | 6 | 47.53 ** |
| Lymphosarcoma and lymphoblastoma | 56 | 36 | 36 | 33 | 14 | 48.69 |
| Glioma of brain | 28 | 27 | 22 | 7 ** | 4 | 40.20 * |
| Other tumors of brain | 47 | 41 | 20 | 27 | 10 | 44.24 * |

* The difference between this percentage, or average, and that for all cancer is statistically significant (P is less than 0.01).
** The difference between this percentage, or average, and that for all cancer is probably statistically significant (P is 0.01 to 0.05). The formulae used for determining statistical significance are given by Schrek (9).

cent of the patients with glioma of the brain. This percentage is based on a small number of patients, but is probably significantly lower than the 26.40 per cent for all cancer, or the 26.70 per cent for all cancer excluding glioma.

*Racial distribution.*—Among the 1,623 primary admissions, 7.94 per cent of the patients were colored (Table III). Patients from the South Atlantic and the East South Central states were colored in a larger proportion of cases (53 and 23 per cent colored respectively). In contrast, groups from the other states had relatively low percentages of colored persons.

The percentage of colored patients with cancer was 7.79; with benign tumors, 9.3. The difference is not statistically significant.

The racial distribution of the patients with various types of cancer is shown in Table II. Carcinoma of the exposed skin, the lip, and the larynx occurred in groups of patients with low percentages colored (0.6, 0, and 2 per cent respectively). In contrast, a high proportion of colored patients (31 per cent) was observed for carcinoma of the esophagus.

*Age.*—The age distribution of the 1,623 patients is shown in Table IV. The ages varied from 18 to 84 years, with an average of 50.14 years. The average ages of patients with benign tumors and with cancer were 47.63 and 50.42 years respectively (Table II). The difference in the average ages is statistically significant. In this hospital, the patients with benign lesions were only slightly younger than those with cancer.

A comparison of the age distribution of white and colored patients was made. The average ages were 50.26 years for the white and 50.67 for the colored patients. The difference is not statistically significant.

Further analysis of the age distribution of white and colored patients with tumors is shown in Table IV where it is seen that between the ages 65 to 84 the percentage colored is low (2.2 per cent), and is significantly lower than the 8.3 per cent colored for patients between the ages 18 to 64.

The average age of the patients with various types of neoplasms is shown in Table II. The highest average age (58.58 years) occurred in the group of men with carcinoma of the prostate. This age is considerably and significantly higher than that for all malignant tumors (50.42 years). Nearly all types of carcinoma, including that of the exposed skin, the lip, mouth, larynx, esophagus, and bladder occurred in patients with fairly high average ages. In sharp contrast, the men with teratoma of the testis had a very low average age (32.90 years). Patients with Hodgkin's disease and with glioma of the brain also had fairly low average ages (40.5 and 40.2 years). In fact all the nonepithelial cancers occurred in patients younger than those with carcinoma.

*Deaths.*—Among the 2,021 cancerous patients admitted or readmitted during 1943, there were 526 deaths in the hospital (Table V). Some of the deaths were due to diseases other than neoplasia. Of the 161 patients with benign tumors, only 4 died. The percentage of deaths was 23.9 per cent for the can-

TABLE III: NUMBER AND PERCENTAGE OF PATIENTS (PRIMARY ADMISSIONS ONLY) RESIDING IN VARIOUS STATES AND IN VARIOUS SECTIONS OF THE COUNTRY

| Residence of patients | Primary admissions | | Percentage of patients | | |
|---|---|---|---|---|---|
| | Number | Percentage | Residing in rural sections | With benign tumors | Colored |
| All states | 1,623 | 100.0 | 25.69 | 9.92 | 7.94 |
| Groups of states | | | | | |
| East North Central states | 831 | 51.2 | 15.9 * | 13.6 | 7.2 |
| West North Central states | 315 | 19.4 | 33.0 * | 6.7 | 5.7 |
| East South Central states | 113 | 7.0 | 43.4 | 5.3 | 23.0 * |
| West South Central states | 280 | 17.3 | 38.6 * | 5.0 | 5.7 |
| South Atlantic states | 17 | 1.0 | 35.3 | 0 | 52.9 * |
| Other states | 67 | 4.1 | 25.4 | 0 | 7.5 |
| Individual states | | | | | |
| Illinois | | | | | |
| Cook County, including Chicago | 304 | 18.7 | 2.0 * | 22.0 * | 12.8 * |
| Other counties | 158 | 9.7 | 38.0 * | 16.5 | 1.9 * |
| Texas | 140 | 8.6 | 35.0 * | 5.7 | 4.3 |
| Ohio | 109 | 6.7 | 11.0 * | 3.7 | 10.1 |
| Indiana | 109 | 6.7 | 21.1 | 6.4 | 2.8 ** |
| Michigan | 96 | 5.9 | 18.8 | 4.2 | 4.2 |
| Missouri | 91 | 5.6 | 23.1 | 5.5 | 14.3 ** |

* The difference between this percentage and that for all states is statistically significant (P is less than 0.01).
** The difference between this percentage and that for all states is probably statistically significant (P is 0.01 to 0.05).

TABLE IV: NUMBER AND PERCENTAGE OF PATIENTS (PRIMARY ADMISSIONS ONLY) IN VARIOUS AGE GROUPS

| Age, years | Primary admissions | | Percentage of patients | |
|---|---|---|---|---|
| | Number | Percentage | Benign tumors | Colored |
| All ages | 1,623 | 100.0 | 9.92 | 7.94 |
| 18 to 19 | 19 | 1.2 | 21 | 0 |
| 20 to 24 | 53 | 3.3 | 19 ** | 8 |
| 25 to 29 | 49 | 3.0 | 8 | 8 |
| 30 to 34 | 41 | 2.5 | 20 ** | 7 |
| 35 to 39 | 30 | 1.8 | 3 | 7 |
| 40 to 44 | 72 | 4.4 | 13 | 8 |
| 45 to 49 | 491 | 30.3 | 10.4 | 9.4 |
| 50 to 54 | 452 | 27.8 | 9.3 | 9.5 |
| 55 to 59 | 202 | 12.4 | 9.4 | 6.4 |
| 60 to 64 | 78 | 4.8 | 9 | 6 |
| 65 to 69 | 92 | 5.7 | 5 | 2 ** |
| 70 to 74 | 34 | 2.1 | 3 | 3 |
| 75 to 79 | 9 | 0.6 | 0 | 0 |
| 80 to 84 | 1 | 0.1 | 0 | 0 |
| 65 to 84 | 136 | 8.4 | 4.4 ** | 2.2 * |

* The difference between this percentage and that for all ages is statistically significant (P is less than 0.01).

** The difference between this percentage and that for all ages is probably statistically significant (P is 0.01 to 0.05).

TABLE V: TYPE OF TREATMENT AND NUMBER AND PERCENTAGE OF DEATHS OF PATIENTS (PRIMARY ADMISSIONS AND READMISSIONS) WITH VARIOUS TYPES OF TUMOR

| | Patients admitted or re-admitted | Treatment | | | | Deaths in hospital | Deaths in hospital, per cent |
|---|---|---|---|---|---|---|---|
| | | Radiation | Surgery | Radiation and surgery | Symptom-atic | | |
| All tumors | 2,204 | 907 | 520 | 223 | 554 | 526 | 23.9 |
| All malignant tumors | 2,021 | 885 | 414 | 217 | 505 | 522 | 25.8 |
| All benign tumors | 183 | 22 | 106 | 6 | 49 | 4 | 2.2 |
| Carcinoma of: | | | | | | | |
| Exposed skin | 306 | 117 | 113 | 13 | 63 | 4 | 1.3 |
| Lip | 111 | 61 | 18 | 6 | 26 | 8 | 7.2 |
| Mouth and tongue | 130 | 67 | 21 | 15 | 27 | 20 | 15.4 |
| Larynx | 111 | 41 | 9 | 29 | 32 | 16 | 14.4 |
| Lung | 158 | 100 | 10 | 18 | 30 | 89 | 56.3 |
| Esophagus | 43 | 9 | 8 | 15 | 11 | 27 | 62.8 |
| Stomach | 90 | 16 | 29 | 14 | 31 | 43 | 47.8 |
| Rectum and rectosigmoid | 101 | 16 | 43 | 17 | 25 | 20 | 19.8 |
| Bladder | 98 | 21 | 42 | 13 | 22 | 31 | 31.6 |
| Prostate | 52 | 6 | 13 | 1 | 32 | 15 | 28.8 |
| Teratoma of testis | 51 | 33 | 0 | 6 | 12 | 14 | 27.5 |
| Hodgkin's disease | 69 | 60 | 0 | 2 | 7 | 22 | 31.9 |
| Leukemia | 93 | 85 | 0 | 0 | 8 | 23 | 24.7 |
| Lymphosarcoma and lymphoblastoma | 56 | 51 | 0 | 0 | 5 | 19 | 33.9 |
| Glioma of brain | 28 | 8 | 7 | 5 | 8 | 15 | 53.6 |
| Other tumors of brain | 47 | 6 | 9 | 3 | 29 | 21 | 44.7 |

cerous and 2.2 per cent for the noncancerous patients.

The number and percentage of deaths for various types of cancer appear in Table V, which shows that of the 43 patients with cancer of the esophagus, 27 (63 per cent) died in the hospital. A large number of deaths were caused also by carcinoma of the lung (56 per cent), stomach (48 per cent), and by glioma of the brain (54 per cent). As would be expected, patients with carcinoma of the exposed skin and carcinoma of the lip had a low percentage of deaths (1.3 and 7 per cent respectively).

The percentage of deaths following each type of treatment was calculated. Radiation in 907 patients was followed by 22.5 per cent deaths, surgery by 16.0 per cent, combined radiation and surgery by 37.2 per cent, and symptomatic treatment by 28.2 per cent. These percentages are of interest but do not, of course, indicate the relative merits of treatment.

Of the 526 patients that died in the hospital, 191 (36.3 per cent) were autopsied.

*Treatment.*—Of the 2,204 patients admitted or readmitted, 907 were treated by radiation, 520 by surgery, and 223 by both (Table V). There were 554 patients who did not require, were unsuited for, or refused radiologic and surgical treatment.

Table VI summarizes the number and types of operations performed on the patients with tumor, and the results. It should be emphasized that this table shows all deaths from whatever cause, including postoperative complications, recurrent cancer, and noncancerous diseases.

It is seen from the table that abdominal-perineal resections of the rectum were performed in 35 patients. These operations were followed by 4 deaths in the hospital and 1 after discharge. Two of the deaths occurred 16 days after the operation, and the other deaths 1, 2, and 5 months after operation. Subtotal gastric resection for carcinoma was performed on 15 patients, only 1 of whom died shortly afterward; another died 8 months later. Tumors of the colon were resected in 17 patients, with no deaths.

TABLE VI: NUMBER AND TYPES OF OPERATION; NUMBER OF DEATHS AND TIME INTERVAL BETWEEN OPERATION AND DEATH

| Operation | No. of patients | Deaths | | Interval between operation and death (each case listed separately) | |
|---|---|---|---|---|---|
| | | In hospital | After discharge | Days | Months |
| *Abdominal surgery* | | | | | |
| Subtotal gastric resection | 15 | 2 | 0 | 4 | 8 |
| Abdominal-perineal resection of rectum | 35 | 4 | 1 | 16, 16 | 1, 2, 5 |
| Resection of colon | 17 | 0 | 0 | | |
| *Thoracic surgery* | | | | | |
| Exploratory thoracotomy | 18 | 7 | 3 | | 1, 1, 3, 3, 4, 4, 5, 5, 6, 6 |
| Lobectomy | 4 | 3 | 0 | | 1, 4, 4 |
| Pneumonectomy | 4 | 2 | 0 | 17 | 2 |
| *Genito-urinary surgery* | | | | | |
| Nephrectomy | 3 | 0 | 0 | | |
| Cystotomy with fulguration of bladder tumor | 34 | 8 | 1 | 2, 25 | 1, 1, 2, 2, 3, 6, 11 |
| Transurethral fulguration of bladder tumor | 26 | 5 | 0 | 13, 15 | 1, 2, 2 |
| Orchidectomy | 5 | 0 | 0 | | |
| Amputation of penis | 5 | 1 | 0 | 8 | |
| *Other surgery* | | | | | |
| Craniotomy only | 14 | 9 | 0 | 0, 0, 1, 1, 1, 3, 6, 14 | 7 |
| Craniotomy with resection of tumor | 15 | 8 | 1 | 1, 1, 1, 1, 3, 14, 17, 19 | 6 |
| Plastic operation on face | 42 | 0 | 0 | | |
| Laryngectomy | 7 | 0 | 0 | | |
| Neck dissection | 28 | 3 | 1 | | 2, 2, 3, 8 |
| Mastectomy | 4 | 0 | 0 | | |

The thoracic surgery included pneumonectomies on 4 patients, 2 of whom died 17 and 68 days respectively following operation. Of 4 patients subjected to lobectomies, 1 is still alive. Thirteen patients had exploratory thoracotomies. Of these 8 died 1 to 6 months after the operation.

Fifteen patients had craniotomies with partial or complete resection of the brain tumor. Eight died within 3 weeks and 1 died 6 months after the operation. Of 14 patients subjected to craniotomies for purposes of relieving intracranial pressure or for exploration, 8 died within 2 weeks and 1 died 7 months after operation.

## DISCUSSION

The administrative value of an annual report from a tumor clinic is obvious and does not require dis-

cussion. Its scientific value is somewhat limited, however, because the statistical data are determined in part by local factors and by the policies of the hospital. It is therefore necessary to evaluate as far as possible these local, extraneous factors that may affect the scientific findings.

*Admissions.*—Men and women who are veterans of a war are entitled to admission to a hospital of the Veterans Administration. If preliminary examinations indicate a tumor, the patient is usually transferred to one of five tumor clinics. In general, hospitals in the Midwest and Southwest transfer their cancerous patients to the tumor clinic at Hines. Patients who are in a terminal condition or who can be treated surgically by the local hospital are usually not transferred. Most of the patients from Illinois and neighboring states enter this hospital directly.

Hines Veterans Hospital has 1,800 beds, of which approximately 500 are allocated to the tumor clinic. This report includes (a) all patients who were admitted or readmitted to the tumor clinic in 1943 with a clinical diagnosis of cancer or benign tumor, and (b) patients who were in the medical and surgical services of the general hospital with a clinical or postmortem diagnosis of cancer. Many of the diagnoses were, of course, substantiated by the microscopic examination of biopsy and surgical specimens.

The patients studied included 2,204 men and 32 women. The small number of the latter is due to the relatively few female veterans.

The data on the relative incidence of tumors among the patients were affected to some extent by selection of the patients transferred by the local hospitals of the Veterans Administration. It is possible that the relative incidence of a few tumors like carcinoma of the stomach and tumors of the brain was underestimated in this study because some of the patients with these tumors were in hopeless condition when admitted to their local hospitals and were therefore not transferred to a tumor clinic. In spite of this extraneous factor, the data on incidence probably have biologic significance. It may be concluded that the most common tumors in veterans residing in the Midwest were carcinoma of the exposed skin, the lung, and the mouth and tongue.

*Geographic distribution.*—Many of the patients from Illinois and the neighboring states were admitted directly to this hospital. Patients from other states usually went first to a local hospital and were transferred to the tumor clinic at Hines if not in hopeless condition and if the lesion could not be treated surgically at the local hospital. This extraneous factor would cause a relative excess of patients from Illinois and the East North Central states. The factor explains the relatively high percentage of benign tumors in patients from the county in which the tumor clinic is located.

Some of the findings on the geographic distribution of patients are probably biologically significant. It was observed that carcinoma of the exposed skin occurred in patients many of whom came from the Southern states and from rural areas. These findings are definitely significant, and are in accord with those of most investigators. In contrast, cancer of the stomach and the testis occurred in patients relatively few of whom were from Southern states. Mountin and Dorn (2) also found that the population of Southern states had a relatively high incidence of cancer of the skin and a low incidence of cancer of the stomach. The role of exposure to sunlight in the etiology of cancer of the skin accounts for this geographic distribution. No explanation can be given, however, for the low incidence of cancer of the stomach and testis among the inhabitants of the Southern states. A remarkably low percentage of patients with glioma of the brain came from rural sections. It is not certain whether this finding is biologically significant or due to extraneous factors.

*Racial distribution.*—The data in this report indicate that carcinoma of the exposed skin and lip occurred with relative infrequency in colored men. This finding is in accord with that of Dorn (1) and other investigators. It was also observed that the colored patients had relatively few tumors of the larynx, but many carcinomas of the esophagus. These observations could not be accounted for by any extraneous factor, and probably are biologically significant.

*Age distribution.*—The population from which the patients were drawn consisted almost exclusively of the veterans of World Wars I and II and of the Spanish-American War. The age distribution of these three groups of veterans was notably different, the minimum ages being approximately 42, 18, and 62 years of age respectively. The heterogeneity of the population in regard to age does not necessarily affect the validity of the findings pertaining to the relative age distribution of patients with different types of tumors.

The most interesting observation was that carcinomas occurred in a higher age group than nonepithelial tumors such as Hodgkin's disease and glioma of the brain. Tumors of the testicle occurred in the youngest age group, with an average age of 32.9 years. It is believed that these findings are biologically significant.

*Deaths.*—The number of deaths in a cancer hospital is dependent upon many factors, such as the patient's alertness in seeking hospitalization and the policies of the hospital in regard to the admission of late cases and the discharge of terminal cases. In

view of these and other extraneous factors the data on the number of deaths in a cancer hospital have little or no scientific value. A comparison of the number of deaths in different institutions would, therefore, be misleading and fallacious. The statistics are, however, interesting and instructive when the percentages of deaths from different types of neoplasia in the same institution are compared.

In this tumor clinic patients are admitted at any stage of the disease, whether early or late. Although no attempt was made to determine the relative numbers of early and late cases, it is known that a large percentage of the patients were in an advanced or terminal stage of the disease. Those in a terminal stage are, for the most part, retained in the hospital except in those few cases where the individual prefers to be sent to his home or to a hospital near it. Other extraneous factors that affect the percentage of deaths arise from the fact that this tumor clinic of 500 beds is part of a general hospital, which has an additional 1,300 beds. Patients whose primary disease was not related to tumor may be referred to the tumor clinic for diagnosis and treatment of a benign or malignant growth. These are included in this report even if the neoplasm was not responsible for death. The report also includes a few cases from the general hospital where a postmortem revealed a cancer that may or may not have been responsible for death.

The various types of tumor had considerably different percentage deaths. The accessible growths, carcinoma of the exposed skin and lip, had the lowest (1.3 and 7.2 per cent). Carcinomas of the mouth and tongue and of the larynx also had fairly low percentages (15.4 and 14.4). The inaccessible tumors of the lung, esophagus, stomach, and brain had high percentage deaths (47.8 to 62.8). Tumors of the testis, although accessible, had a fairly high percentage (27.5), evidently because of their great malignancy.

*Value of an annual report from a tumor clinic.*—One of the chief difficulties in the evaluation of statistical data on cancer has been the lack of controls. For example, Schrek and Gates (7) found that the median age of patients with epidermoid carcinoma of the skin was 67.2 years. This figure by itself was meaningless, because it did not show whether cutaneous carcinoma had a tendency to develop in an older age group or whether the particular hospital (Pondville Hospital) obtained its patients from a population of old people. Similarly Steiner (9) found on analysis of necropsies at the University of Chicago that cancer of the lung increased in incidence from 1902 to 1941. A control group was needed to determine whether the increase was biologically significant. It is evident that statistical data, like experimental data, require suitable control groups.

Various controls have been utilized. The statistical data on a group of cancer patients can be compared with analogous data on all the patients in the hospital. This method may be adequate for some studies, but in general it is unsatisfactory because all the patients in a general hospital form a highly heterogeneous group.

A second, more satisfactory, control is the patients with a different type of cancer. In the example cited previously, the median age of the patients with epidermoid carcinoma was compared by Schrek and Gates (7) with the median ages for basal cell carcinoma and for cancer of the breast. Steiner (9) compared the incidence of cancer of the lung in necropsies at the University of Chicago with the incidence of four other types of tumor. This type of control is frequently satisfactory if the control group is chosen with care. It may be difficult, however, to eliminate the investigator's preconceptions and bias in the choice of a tumor to be used as a control.

A third control group may be established by using all patients with cancer. One thus avoids the dangers and difficulties of choosing any particular type of tumor as a control. Schrek (5) used such a group in a study on cutaneous carcinoma in the Negro.

In a later investigation he (6) observed that the statistical data for all tumors were inordinately affected by two or three types of growths. He therefore set up a new control group, which consisted of all patients with cancer except those with certain types of tumors. This method seems to be satisfactory because (a) the control group varies in different studies and depends on the subject under investigation, and (b) the control group is determined by well defined rules and is not determined by the investigator's preconceived ideas.

To set up a suitable control group it is necessary to make a complete survey of the cancer patients. This is the primary function of an annual report from a tumor clinic. It serves as a guidepost, which is necessary in the evaluation of all other statistical reports from the clinic. In addition to serving as a control to future investigations the annual report, in itself, yields interesting statistical data on such problems as the racial, geographic, and age distribution of the patients with cancer. An important function of the annual report is that it suggests further lines of statistical investigation. For example, this paper raises the question whether glioma of the brain is more prevalent in urban than in rural populations. It may be concluded that a detailed annual report of a large tumor clinic has definite scientific usefulness.

The value of annual reports would be greatly enhanced if many tumor clinics prepared and published detailed reports on a uniform basis.

A comparison of such reports would indicate which findings are due to local conditions and which are biologically significant. Complete reports from all the tumor clinics in the country would delineate quite sharply the problem of cancer in the United States.

### The Importance of an Independent Statistical Department

The preparation of an annual report from a tumor clinic and the setting up of adequate control groups for cancer statistics necessitate an independent statistical department. The collection of statistics by each clinical department is unsatisfactory because (a) such an organization lacks a unifying force in the collection and analysis of statistics; (b) many statistical problems pertain to several clinical departments; and (c) the analysis of data requires specialized training in statistical methods. The pathologist, the roentgenologist, and the statistician utilize entirely different tools and each requires an independent department. Furthermore, a statistical department is needed for the training of men in cancer statistics and for the development of new statistical methods. It may be concluded that effective cancer statistics can be obtained only with an efficient independent organization for the collection, compilation, and analysis of the data.


### SUMMARY

During 1943 the tumor clinic admitted 1,623 patients for the first time, and readmitted 581 patients. This report presents a detailed analysis of the racial, geographic, and age distribution of the patients with various types of neoplasia. The type of treatment administered and the number of deaths in the hospital are also considered.

A relatively high percentage (51 per cent) of the patients with carcinoma of the exposed skin came from Southern states, but low percentages (17 and 12 per cent) with cancer of the stomach and testis were also from the South.

Patients with carcinoma of the exposed skin and lip were relatively often rural, but few with glioma of the brain came from rural districts.

Carcinoma of the exposed skin, lip, and larynx occurred relatively more often in white than in colored patients, but carcinoma of the esophagus occurred relatively more often in the colored.

The average age of the patients was 50 years, but those with cancer of the testis, glioma of the brain, and Hodgkin's disease had a lower average age (36 to 41 years).

The percentage of deaths for all patients was 24 per cent. Carcinoma of the esophagus had the highest percentage of deaths (63 per cent) and carcinoma of the exposed skin the lowest (1.3 per cent).


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# Note to the Editor

An abstract of a paper by Hanhart on the "Surprisingly Slight Importance of Heredity in Cancer as Indicated by Very Frequent Freedom from the Disease among Progeny of 121 Cancerous Married Couples in Canton Glarus" * has recently appeared in *Cancer Research* (5:189. March 1945). Some comment on the method of analysis employed and the conclusions drawn by the author seems advisable.

In the records of Canton Glarus, Switzerland, the author found 141 instances in which both husband and wife had had cancer. He excluded from these 13 childless couples, and 7 couples all of whose offspring were still under 40 years of age at the time of study. The remaining 121 marital couples had 590 children, of whom 359 were over 40 years old. Offspring who had died of causes other than cancer previous to the time of study were apparently not considered, but the data presented do not permit accurate conclusions on this point. Among the 359 offspring over 40 years old, 41 cases of cancer had already occurred; among the 286 over 50 years old there were 38 cancer cases, or 13.3 per cent. This percentage is compared with the total expectancy of acquiring cancer among males over 45 years old in the city of Zurich, a figure stated to be 21 per cent on the authority of Schinz and Senti (Zürich. Statistischen Nachrichten, 3. 1932). This latter paper is not available to the present writer; one would like to know whether the percentage given is the expectancy of developing cancer or of dying of it (the German word used is "Krebserwartung"—cancer expectation) and whether the mortality rates were resident or recorded, since the latter, in a hospital center such as Zurich, would be considerably higher than the true ones.

Even if one accepts 21 per cent as the normal expectancy in Switzerland of developing cancer from 45 years on, what Hanhart is comparing with it is the accumulated cancer mortality (incidence?) among persons who have already attained various ages over 50, which is a quite different matter. For example, according to the cancer mortality rates for upstate New York, 1939 to 1941, by the time age 50 is reached slightly less than 1 per cent (0.92 per cent) of males will have died of cancer but the *expectancy* of dying of cancer from age 50 on, for males, is 12.3 per cent. These two probabilities, in the upstate New York statistics, do not equal each other in either sex until the age group 75 to 79 years is reached, and one would expect the Swiss data to be similar.

It is possible that Hanhart's figure for accumulated cancer incidence (or is it mortality?) among these offspring of cancerous parents is too high because he has failed to count all that were born to these parents. On the other hand, if it is correct it would suggest a higher cancer incidence than normal. At any rate, the analysis as presented is insufficient to determine whether the cancer incidence among these offspring differs from what would be expected, and does not permit any conclusion on this point. Hanhart neglects entirely the fact that the offspring alive at the time of his study had about 15 years more of life (estimated approximately from Swiss life tables) and about half the total average risk of developing cancer yet to go through.

Of 121 marital couples, both of whom died of cancer, in 33 pairs, or 27.3 per cent, both had cancer of the stomach. The average age of these cancerous spouses is given as 66 years. For the years 1911 to 1920 the cancer deaths in the 60 to 69 years age group in Switzerland show 45.7 per cent attributed to stomach cancer in males and 39.7 per cent in females. The product of these probabilities is 18.1 per cent, which is significantly less than the 27.3 per cent concurrence found. This suggests marital association for site of cancer, a possibility which, though not discussed by the author, may merit further consideration by him.

Even more striking is the fact that where both parents had stomach cancer, out of 15 offspring over 40 years of age who developed a malignant tumor the site was also the stomach in 11, or approximately three-fourths. This is stated by the author to be a smaller incidence of stomach cancer than expected! On the contrary, it points strongly to organ selection for localization of cancer in families in which stomach cancer occurred in the parents. This observation would agree with those of Waaler, Wassink, and Deelman.

It is regrettable that basic data as interesting as these, no doubt gathered at considerable labor and expense, should have received analysis insufficient to test the points at issue or to support the conclusions advanced.

MORTON L. LEVIN, M. D.

* **Auffallend geringe Bedeutung der "Belastung mit Krebs" bewiesen durch das sehr häufige Freibleiben der Nachkommen aus 121 Ehen krebskranker Gatten im Kanton Glarus.** HANHART, E. *Schweiz. med. Wchnschr.*, **73**:446-449. 1943.

# Abstracts

## Reports of Research

**Acceleration by Means of Prolonged Mechanical Irritation of Carcinogenesis in the Skin of Mice Painted with 1:2:5:6-Dibenzanthracene.** RILEY, J. F., and PETTIGREW, F. W. [Edinburgh Univ., Edinburgh, Scotland] *Brit. J. Exper. Path.*, **26**:63-66. 1945.

Two hundred inbred mice were painted once weekly for 16 weeks with a 3% solution of 1,2,5,6-dibenzanthracene in acetone and divided into 2 groups: 'A' (93 mice) were then brushed twice weekly with a small brush of stiff bristle—a form of mechanical irritation which preliminary experiments had shown to be unassociated with surface ulceration; 'B' (92 mice) remained as a control. After 33 weeks the mechanical irritation was stopped, and both groups were painted with the carcinogen twice weekly for a further 16 weeks.

"Papillomas began to appear in both groups of mice within 2 weeks of [first] cessation of painting; for the next 6 weeks there was no significant difference between the 2 groups. Thereafter the numbers of both papillomas and carcinomas were significantly higher in the brushed group 'A' than in the controls 'B.' At the 49th week 55 mice in Group 'A' had borne tumours, of which 13 were malignant; while in Group 'B' the number of papillomas was 36, of which only 4 had undergone malignant transformation. The additional factor of the mechanical trauma in Group 'A' must be held responsible for this difference in tumour emergence in the two groups.

"On the resumption of painting, fresh papillomas appeared in both groups of mice, while several pre-existing papillomas which had become stationary took on rapid and invasive growth. . . . At the end of the experiment the total number of carcinomas (9) in the control group was less than the number of carcinomas (13) in the brushed group at the 49th week. The brushed group finally yielded a total of 21 carcinomas."—I. H.

**The Change from Benign to Malignant in Chemically Induced Warts in Mice.** MOTTRAM, J. C. [Mt. Vernon Hosp., Northwood, Middlesex, England] *Brit. J. Exper. Path.*, **26**:1-4. 1945.

The author has compared the structure of papillomas produced by (a) multiple paintings with 3,4-benzpyrene and (b) a single painting accompanied by application of croton oil. He considers that benzpyrene acts especially on proliferating cells. "In benign warts, especially those growing rapidly, proliferation is proceeding more rapidly than in conditions of hyperplasia, and therefore benign warts would be expected to be very sensitive to the production of new tumours in them by further paintings of benzpyrene. New benign warts in existing benign warts are likely to remain undetected; on the contrary, new malignant tumours will soon manifest themselves. Thus is visualized, not a step-up process, but repeated hits by the benzpyrene on a sensitive tissue, any one of which is capable of producing, and may result in a malignant tumour."—E. L. K.

**The Histogenesis of Benzpyrene-Induced Epidermal Tumors in the Mouse.** GLÜCKSMANN, A. [Strangeways Research Lab., Cambridge, England] *Cancer Research*, **5**:385-400. 1945.

The effects, on the mouse and rat skin, of a single and of repeated weekly paintings with benzpyrene (1% in acetone) for periods ranging from 30 minutes to 15 months are analyzed by a quantitative histological method based on the classification and counts of cells in selected comparable areas. Quantitative control observations on the normal postnatal development of the mouse epidermis and on its reaction to acetone painting, to chemical depilation, and to repeated weekly paintings with turpentine are also recorded.

In the normal mouse epidermis the hair follicles act as germinative centers, and the epidermal hyperplasia induced by chemical depilation is accounted for by cellular migration from, and greater mitotic activity in, the hair follicles. The regenerative hyperplasia following repeated turpentine paintings is due, at first, to cellular migration and greater mitotic activity in the hair follicles, and, subsequently, to increased proliferation of the epidermal cells themselves. The hyperplasia of the mouse epidermis and of its hair follicles induced by a single application of benzpyrene is due to a direct increase of the mitotic activity in the epidermis itself as well as in the hair follicles. These proliferative changes are not secondary to primary deleterious effects since there is only a minor increase in the number of disintegrating cells, no early ulceration, and no evidence of a specific growth inhibition. The epilation is secondary to increased proliferative activity in the hair follicles.

The rat epidermis fails to react to benzpyrene paintings and does not produce epidermal hyperplasia or tumors.

The primary reactions of the mouse epidermis to benzpyrene are generalized over the treated area and consist of: (1) an increase in cell growth (involving the number as well as the size of cells), and (2) a delay in the onset of differentiation. These reactions result in an absolute and relative increase in the number of resting cells (*i.e.* undifferentiated cells capable of division), and a relative decrease in the number of keratinizing cells, which together quantitatively account for the epidermal hyperplasia and the formation of papillomas. Carcinomas develop in hyperplastic or in papillomatous regions. Their appearance is characterized by a further absolute and relative increase in number of the now anaplastic resting cells, by a quantitative and qualitative disturbance

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of the differentiation process, and by the induction of a focal inflammatory reaction. The bearing of these findings on the interpretation of carcinogenesis in the mouse epidermis is discussed.—Author's abstract.

**Kernveränderungen an Rattenlebern unter Einwirkung des cancerogenen Stoffes 4-Dimethylaminoazobenzol (Buttergelb). [Nuclear Changes in the Rat Liver Produced by the Carcinogen 4-Dimethylaminoazobenzene (Butter Yellow).]** Langer, E. [Path. Inst., Deutsche Karls-Univ., Prague, Czechoslovakia] *Ztschr. f. Krebsforsch.,* **52**:443-454. 1942. From abstr. in *Chem. Zentralbl.,* **1**:284. 1943.

Nuclei with double volume (rhythmic growth in the sense of Jacobjs—*Roux Arch.,* **106**:124. 1925) appeared as the first morphological change in the liver of rats fed *p*-dimethylaminoazobenzene. These large nuclei then gave way to irregularly increased nuclei. Adenomas arising after cirrhosis may show a variation in nuclear size, which in its rhythm recalls the early stage of *p*-dimethylaminoazobenzene action and bears witness to the autonomy of the tumor-like growth. Since a nuclear increase (doubling of nuclear volume) has been observed also after the entry of other substances into the liver, it cannot be regarded as a specific criterion of the action of carcinogenic substances.—M. H. P.

**Changes in Epidermal Cholesterol during Methylcholanthrene Carcinogenesis in Mice.** Wicks, L. F., and Suntzeff, V. [Barnard Free Skin and Cancer Hosp., and Washington Univ., St. Louis, Mo.] *Cancer Research,* **5**:464-468. 1945.

The total cholesterol content was determined in the separated epidermis of normal mice and of mice that had received applications of methylcholanthrene in benzene. The values found for cholesterol are expressed as a ratio in relation to the protein N content of the epidermis. A single application was sufficient to produce a profound lowering of this ratio. Subsequent applications during the first 60 days produced only a further slight reduction. A single application of benzene alone greatly increased the cholesterol/protein N ratio of the epidermis. Repeated applications of benzene diminished this ratio to values somewhat below the normal level but higher than the values observed in skin exposed to methylcholanthrene. The effect of methylcholanthrene in reducing the values for the cholesterol/protein N ratio resembled closely its effect on the total lipid/protein N ratio recorded in a previous paper.—Authors' abstract.

**The Effect of 3,4-Benzpyrene on the Autoxidation of Unsaturated Fatty Acids.** Mueller, G. C., and Rusch, H. P. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Cancer Research,* **5**:480-484. 1945.

The effect of 3,4-benzpyrene on the autoxidation of purified linoleic and linolenic acids at 37° C. was investigated by adding varying amounts of the hydrocarbon and determining the oxygen uptake, the iodine number, and the peroxide value of the mixtures at regular intervals. The rate of disappearance of the carcinogen from autoxidizing linoleic acid was also measured under similar conditions. The data demonstrate that the autoxidation of these acids was inhibited by all levels of benzpyrene employed, but large amounts of the hydrocarbon (3.2%) were more effective than small amounts (0.2%); however, the degree of inhibition was not directly proportional to the level of carcinogen employed. Benzpyrene was destroyed at a rate that was proportional to the speed of fatty acid oxidation. Only a small part of the carcinogen that had disappeared could be accounted for in the form of the mixed quinones of benzpyrene. 3,4-Benzpyrene-5,8-quinone also inhibited the autoxidation of linoleic acid and also was destroyed during the process.

The evidence indicates that 3,4-benzpyrene as well as the mixed quinones of this hydrocarbon undergo a coupled oxidation when dissolved in an autoxidizing unsaturated fatty acid.—Authors' abstract.

**Untersuchungen über die Rolle der Porphyrine bei geschwulstkranken Menschen und Tieren. [The Role of Porphyrins in Tumor-Bearing Persons and Lower Animals.]** Auler, H., and Banzer, G. [Univ.-Klin. f. Geschwulstkranke, Berlin, Germany] *Ztschr. f. Krebsforsch.,* **53**:65-68. 1942. From abstr. in *Chem. Zentralbl.,* **1**:1174. 1943.

Most patients with carcinoma showed porphyrin excretion that was above normal and that did not parallel pathological urobilin or urobilinogen excretion. Ulcerated tumors, necrotic areas especially, fluoresced in ultraviolet light, and this property was intensified by injections of Photodyn (hematoporphyrin). Rats bearing Flexner-Jobling carcinoma or Jensen sarcoma and injected with porphyrin showed an increase in porphyrin content around the tumors. Even without porphyrin injections, ulcerated tumors contained porphyrin as shown by extraction and spectroscopic determination.—M. H. P.

**Distribution of Radioactive Sulfur in the Rat.** Singher, H. O., and Marinelli, L. [Memorial Hosp., New York, N. Y.] *Science,* **101**:414-415. 1945.

Heptaldehyde bisulfite containing $S^{35}$, injected intraperitoneally, accumulated in rat lymphosarcoma to approximately the same extent as it did in normal tissues such as thymus, spleen, lymph node, kidney, liver, and brain. Hair and bone matrix contained about twice as much $S^{35}$ as did the tissues mentioned above, while bone marrow contained by far the largest amount of all. Tests with sodium sulfate and cinnamaldehyde bisulfite both containing $S^{35}$ indicated that the distribution of sulfur in certain tissues (no data for lymphosarcoma) is somewhat dependent upon the chemical structure of the compound. However, irrespective of the compound used, the highest concentration of $S^{35}$ occurred in the bone marrow.—R. B.

**Diagnosis of Primary Cancer of the Liver.** Schoental, R. [Brit. Emp. Cancer Campaign Research Centre, Sir William Dunn Sch. of Path., Oxford, England]. Letters to the Editor. *Lancet,* **248**:226-227. 1945.

The author refers to an investigation carried out at the University Clinic in Cracow and published in a Polish journal (*Polska gaz. lek.,* **17**:1011. 1938) which is not now accessible. Estimations of lactic acid in the blood showed normal values (9-15 mgm. per 100 ml.) with occasional variations up to 100%, in most of the diseases studied, including cases of cancer of which some showed extensive metastases in the liver. Three cases of primary carcinoma of the liver gave very much higher values (60-130 mgm. per ml.). Possible application to differential diagnosis of primary and secondary tumors of the liver is suggested.—E. L. K.

**The Fate of Estrogenic Metahormones in the Liver.** Lipschütz, A., Becker, C., Mello, R. F., and Riesco, A. [Nat. Health Service, Santiago, Chile, and São Paulo Univ., São Paulo, Brazil] *Science,* **101**:410-411. 1945.

Pellets of estriol, equilenin, α- and β-dihydroequilenin, implanted into the spleen of castrated female guinea pigs and thus absorbed via the hepatic circulation, were much less fibromatogenic than they were when implanted subcutaneously. From this it was concluded that these urinary estrogens or metahormones must have been chemically altered in the liver, and thus could not represent the final products of ovarian estrogen inactivation by liver.—R. B.

**Castration Effects of the Inherited Hormonal Influence.** Smith, F. W. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Science,* **101**:279-281. 1945.

Female mice of the A and Z (C3H) strains differed strikingly in their reproductive physiology following ovariectomy at an early age (4 weeks). In the Z mice, the vagina opened at 6 weeks, and the majority of mice displayed estrus at 5 to 7 months. There was hyperplasia of the adrenal cortices, uteri were large and cystic, and mammary glands showed extensive duct development, signs of active growth in the end bulbs, and precancerous and cancerous nodules. Castrated A × Z hybrids displayed all of these evidences of hormonal stimulation when they carried the milk agent; in the absence of the milk agent they showed the same changes with the exception that no precancerous nodules developed. By contrast, in ovariectomized mice of the A strain, the vagina did not open until the animals were 7 months old, and at this age none displayed estrus. The uteri were small, the majority of adrenal cortices were not hyperplastic, the mammary glands varied considerably in extent of growth but had no precancerous lesions.

These differences in reproductive physiology following ovariectomy are correlated with an inherited difference in incidence of mammary cancer among normal virgin females, the Z strain having a high incidence and the A strain a low one. This difference in incidence is interpreted as being due to a hormonal influence. In the ovariectomized Z mice, it is thought that the hormonal influence brings about hyperplasia of the adrenal cortex, which in turn produces changes in uteri and mammary glands, as described above. In the A strain, this hormonal influence is thought to be reduced or absent.—R. B.

**The Role of Nutrition in Cancer Prevention.** Potter, V. R. [Med. Sch., Univ. of Wisconsin, Madison, Wis.] *Science,* **101**:105-109. 1945.

Recent work has shown that mice maintained on low calorie diets and having increased muscular exercise develop cancer less readily than do control mice. It has also been shown that chemical or physical irritation can augment the effects of carcinogenic agents administered in liminal or subliminal doses. In the present article the author gives an enzymological interpretation of these facts, and also states that they provide us with a definite guide toward the prevention of cancer in human beings. Cancer prevention, he suggests, may consist in eating no more than we need of a carefully selected diet, in keeping physically fit, and in eliminating chronic irritations.—R. B.

**Ample Exercise and a Minimum of Food as Measures for Cancer Prevention?** Morris, H. P. [Nat. Cancer Inst., Bethesda, Md.] *Science,* **101**:457-459. 1945.

Morris believes that evidence from animal experimentation does not as yet warrant the adoption of restriction of food intake and increased exercise as measures for the prevention of cancer in man—as advocated by Potter [see preceding abstract]. Evidence concerning the effect of exercise is scanty and not clear cut. With regard to diet, the evidence of reduction in cancer incidence with decreased food intake is derived largely from experiments on mammary cancer in mice, and there is no reason at present to assume that the majority of other types of cancer would be affected similarly. In fact, nutritionally poor diets are known to favor the development of liver cancer in rats given *p*-dimethylaminoazobenzene. It is also pointed out that in the case of mammary cancer the dietary regimens that bring about reduction in cancer incidence also retard body growth or cause loss of weight in adults; these regimens inhibit estrus and mammary gland development. Such a treatment, even if applicable to human beings, would be too drastic. Although it is possible that a relatively small reduction of food intake might bring about a reduction in cancer incidence, or delay its appearance, there is at present no experimental evidence for this view, and the author concludes that no generalizations are possible with regard to diet and cancer.—R. B.

**Influence of the Mucoprotein of the Serum of Immunized Fowls on Development of Avian Leukemia.** Marchal, S., Paturel, L., Guérin, M., and Guérin, P. *Compt. rend. Soc. de biol.,* **137**:62-63. 1943. From abstr. in *Chem. Abstr.,* **39**:2324-2325. 1945.

The serum mucoid fraction from fowls immunized against leukemia contains an antibody, and, when injected intramuscularly into fowls inoculated with blood from a leukemic bird, delays but does not prevent death from this disease.—M. H. P.

**Application of the Chromate Treatment of Dr. Hasse to Cancer in Mice.** Regamey, J. *Bull. Soc. vaudoise d. sc. nat.,* **61**:403-410. 1941. From abstr. in *Chem. Abstr.,* **39**:2333. 1945.

Subcutaneous injections of 0.1 cc. of 0.001 to 0.0001 *M* potassium dichromate 16 to 20 times in the course of 2 to 3 days had no beneficial effect in mice with inoculated tumors, benzpyrene cancer, or spontaneous mammary carcinoma. A 0.005 *M* solution was toxic to the mice.—M. H. P.

**Treatment of Cancer in Mice.** Regamey, J. *Bull. Soc. vaudoise d. sc. nat.,* **62**:119-134. 1942. From abstr. in *Chem. Abstr.,* **38**:2745. 1944.

Inhibition or regression of tumors followed the feeding of dried organs (pancreas, stomach, intestine, adrenals, bone marrow, testes, or hypophysis) with amino acids (*d*-alanine, cystine, proline, and glutamic acid) to mice with spontaneous mammary cancer or with tumors induced by benzpyrene or methylcholanthrene. However, the treatment did not prolong the survival period.—M. H. P.

# Clinical and Pathological Reports

*Clinical investigations are sometimes included under Reports of Research*

## Skin and Subcutaneous Tissues

**Further Studies on the Mode of Origin of Carcinomas of the Skin.** Willis, R. A. [Alfred Hosp., Victoria, Australia] *Cancer Research,* **5**:469-479. 1945.

Carcinomas of the skin of most diverse types arise from fields of epithelium of greater or less extent. The areas of origin of the most circumscribed tumors are often 1 or 2 cm. in diameter. Carcinomas of scars and chronic ulcers and other precancerous inflammatory lesions may arise simultaneously or successively over extensive areas of epidermis. Progressive cancerous change in areas of predisposed epithelium is often demonstrable in early tumors; it may commence at one focus or at multiple foci. If a tumor is excised from a field of predisposed epidermis in which cancerous change is still in progress, recurrence of growth may take place by neoplastic conversion of residual parts of the field. The still prevalent view that tumors usually arise each at a single point in time from a single small focus is false; it is but an echo of Cohnheim's and Ribbert's views, and should cease to influence modern pathologists. [These conclusions are based on a study of numerous specimens from the human subject.]—Author's summary.

**Giant Nevus of the Thigh Successfully Treated by Complete Excision and Primary Grafting.** Pickrell, K. L., and Clay, R. C. [Johns Hopkins Hosp. and Univ., Baltimore, Md.] *Arch. Surg.,* **48**:319-324. 1944.

A nevus measuring 19 × 10 inches was removed from the lower extremity of a 6 year old boy, and the defect covered with split thickness grafts obtained from the posterior surface of the trunk. The grafts healed by first intention.—W. A. B.

**Multiple Rodent Ulcers.** Glen, E. M. *Proc. Roy. Soc. Med.,* **38**:127. 1945.

Description of a case.—E. L. K.

## Eye

**Diagnosis of Orbital Tumors. Address of the Guest of Honor [American Medical Association Annual Session 1944, Section of Ophthalmology].** Benedict, W. L. [Mayo Clin., Rochester, Minn.] *J. A. M. A.,* **126**:880-884. 1944.

Exophthalmos in the absence of hyperthyroidism is strongly suggestive of an expanding lesion in the orbit. Exploration of the orbit for diagnosis should always be done through an opening large enough and so placed that should a tumor or any other pathological condition requiring surgical removal be found, the operation can be completed at the time.—M. E. H.

**Bilateral Metastatic Carcinoma of the Choroid with X-Ray Therapy to One Eye.** Cordes, F. C. [Univ. of California Med. Sch., San Francisco, Calif.] *Am. J. Ophth.,* **27**:1355-1370. 1944.

A review of the subject of metastatic carcinoma of the choroid with a report of a case in which the disease occurred in both eyes. One eye was enucleated, and microscopic sections revealed the tumor to be scirrhous adenocarcinoma. The primary growth was in the breast. The remaining eye was treated with x-ray, and good vision was retained until the patient's death 23 months later. A long bibliography is included.—E. C. R.

## Breast

**Primary Carcinoma of Both Breasts.** Buford, R. K., and Iatesta, M. A. [Charleston, W. Va.] *West Virginia M. J.,* **40**:386-387. 1944.

A case report. A scirrhous carcinoma was removed by radical procedure from the breast of a 32 year old woman. Five years later a similar tumor, thought to be another primary scirrhous carcinoma, was removed with the other breast. The patient was living at the time of the report; there was no clinical evidence of metastases. —E. E. S.

**Chronic Cystic Mastitis: With Particular Reference to Classification.** Cole, W. H., and Rossiter, L. J. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Ann. Surg.,* **119**: 573-590. 1944.

Chronic cystic mastitis is divided into four categories based on gross and microscopic appearance. Adenofibrosis and cystic disease are least likely to be forerunners of carcinoma. Although benign parenchymatous hyperplasia and precancerous hyperplasia show no invasion, there is the likelihood that cancer may develop in breasts with such lesions, particularly in the latter instance, in which proliferation of cells results in layering and plication. Simple mastectomy is indicated when local excision reveals precancerous hyperplasia.—W. J. B.

## Female Genital Tract

**The Relationship of Granulosa Cell Tumors of the Ovary to Endometrial Carcinoma.** Ingraham, C. B., Black, W. C., and Rutledge, E. K. [Univ. of Colorado Sch. of Med., Denver, Colo.] *Am. J. Obst. & Gynec.,* **48**:760-773. 1944.

Two additional cases of granulosa-cell tumor and one of theca-cell tumor of the ovary associated with adenocarcinoma of the corpus uteri are reported. The role of the ovaries in carcinoma of the uterus is discussed. It is pointed out that endometrial carcinoma and granulosa- and theca-cell ovarian tumors are often associated. The question of definition of cancer of the corpus uteri is reviewed.—A. K.

**The Spread of Uterine and Ovarian Carcinoma With Special Reference to the Rôle of the Fallopian Tube.** Lynch, R. C., and Dockerty, M. B. [Mayo Foundation, and Mayo Clin., Rochester, Minn.] *Surg., Gynec. & Obst.,* **80**: 60-65. 1945.

A study was made of 113 cases of uterine and ovarian carcinoma in which the malignant process had invaded other pelvic structures. Direct extension and lymphatic permeation singly or together were the chief ways of dissemination, with spread through the lumen of the fallopian tube occurring in a few cases.—J. G. K.

**Recognition and Management of the Woman Predisposed to Uterine Adenocarcinoma.** RANDALL, C. L. [Univ. of Buffalo, and Buffalo Gen. Hosp., Buffalo, N. Y.] *J. A. M. A.*, **127**:20-25. 1945.

The high incidence of adenocarcinoma occurring prior to the menopause demands that adequate investigation and treatment be carried out whenever abnormal bleeding occurs in a woman more than 40 years of age. When postmenopausal bleeding occurs, the woman with a history of menorrhagia during the climacteric has a 3½ times greater chance of having developed cancer than does the woman who experienced no increased bleeding before the cessation of her periods. There is too little general recognition of the fundamental role played by the ovary in the development of adenocarcinoma of the uterus. The author believes that the woman whose estrogenic stimulation stops at the menopause is not likely to develop adenocarcinoma of the uterus.—M. E. H.

**Cancer of the Cervix.** MORGAN, H. W. [Mason City, Iowa] *J. Iowa M. Soc.*, **35**:6-8. 1945.

Adequate biopsy of suspicious lesions is urged.—M. E. H.

## MALE GENITAL TRACT

**Androgen Control Therapy in 130 Cases of Carcinoma of the Prostate.** HERGER, C. C., and SAUER, H. R. [New York State Inst. for Study of Malig. Dis., Buffalo, N. Y.] *Surg., Gynec. & Obst.*, **80**:128-138. 1945.

A detailed analysis of 130 patients treated with castration, stilbestrol, or both, indicated that a favorable response was accomplished in numerous instances though the improvement was not sustained in a considerable proportion.—J. G. K.

**How Should Cancer of the Prostate be Treated?** WRIGHT, B. W. [Nashville, Tenn.] *J. Tennessee M. A.*, **38**: 35-39. 1945.

The author states that in his experience less than 10% of prostatic cancers are treated early enough to be eradicated by operation. He deems it unwise to castrate and administer estrogens until severity of symptoms warrants palliative procedure; estrogens should be administered in sufficient quantities to produce gynecomastia, and then in maintenance dosage.—E. E. S.

**Teratoma of the Testicle. Metastasis to the Epigastrium Treated by Bilateral Orchidectomy—Recovery.** SALEEBY, E. R. [Jefferson Hosp., Philadelphia, Pa.] *Ann. Surg.*, **119**:262-265. 1944.

The patient also received irradiation and is alive 14 months after bilateral orchidectomy.—W. J. B.

**Cystoma of Seminal Vesicle.** PLAUT, A., and STANDARD, S. [Beth Israel Hosp., New York, N. Y.] *Ann. Surg.*, **119**:253-261. 1944.

A case in which the tumor was removed through an abdominal incision is reported in detail, and a discussion of the literature is added.—W. J. B.

**A Clinic on Carcinoma of the Penis.** SAUER, D., and LEIGHTON, W. E. [Barnard Free Skin and Cancer Hosp., and St. Louis Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**:1211-1219. 1944.

The authors review 138 cases seen at the Barnard Free Skin and Cancer Hospital between 1906 and 1944, and give results for various types of treatment.—J. L. M.

## URINARY SYSTEM—MALE AND FEMALE

**Urine Sediment Smears as a Diagnostic Procedure in Cancers of the Urinary Tract.** PAPANICOLAOU, G. N., and MARSHALL, V. F. [Cornell Univ. Med. Coll., and New York Hosp., New York, N. Y.] *Science*, **101**:519-520. 1945.

Smears of centrifuged urine sediments from 83 patients (70 men, 13 women) were studied. On the basis of the cytology of the smears, 27 were classified as positive for neoplasm. In 24 of these (89%), the smear diagnosis was confined by biopsy (21 cases) or clinically (3 cases). In the remaining 3 cases, the final diagnosis was uncertain. There were no definitely false positive results. In the group of cases in which the smears were negative or inconclusive, the proportion of correct diagnosis was about 60%. These results prove that neoplasms located in organs of the urinary tract undergo superficial desquamation of a proportion adequate to allow diagnosis by urine sediment smears, although the type and location of the tumors are not always clear. The method is still being developed, and the authors state that it should not be used for final diagnosis without corroboration by other means.—R. B.

**Clinical-Pathological Review of Tumors of the Urinary Tract with Relationship of Age and Sex Incidence and Functional Disturbances.** BLEYER, L. F. [Evanston, Ill.] *Urol. & Cutan. Rev.*, **48**:516-522. 1944.

A general article—V. F. M.

**Classification of Renal Neoplasms: A Clinical and Pathological Study Based on 199 Cases.** MELICOW, M. M. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *J. Urol.*, **51**:333-385. 1944.

Among the 199 cases of renal neoplasm at Squier Urological Clinic, the incidence was higher in males than in females, and was the same in the right as in the left kidney, but the incidence of inoperable neoplasms and of highly malignant mixed tumors was much higher in the left. Upon a detailed study of histological specimens, the author builds a classification of renal neoplasms. True Grawitz tumors are rare, and the common types of neoplasm are thought to arise from cells of the adult kidney. Radiographic diagnosis and prognosis for various types of kidney tumors are discussed.—V. F. M.

**Classification of Tumors of the Kidney.** FITE, G. L. [Nat. Inst. of Health, Bethesda, Md.] *Arch. Path.*, **39**:37-41. 1945.

A study of more than 600 renal tumors has led the author to conclude that the tumors derived from the adult renal parenchyma all have a basic papillary structure, although some may be solid and others may be partly papillary and partly solid. About half the tumors could be placed in one or another of 4 groups representing 4 distinct cellular and structural types. Cells of adrenal origin seemed to play no part in the histogenesis of the renal tumors studied.—J. G. K.

**Primary Tumors of the Kidney.** SHAHEEN, A. L., CASSANO, C., and LISA, J. R. [City Hosp., New York, N. Y.] *J. Urol.*, **51**:597-600. 1944.

Thirty renal carcinomas were found in 5,100 necropsies; 66% were in patients more than 50 years of age. The incidence was about 3 times greater in males than in

females. Metastasis by way of the lymphatics or blood vessels was common, the former route predominating. Forty-six benign adenomas and 1 sarcoma also were found.—V. F. M.

**The So-Called Grawitz Tumor and Its Interpretation by Help of a New Concept of Heterotopic Tumors.** SCHILLER, W. [Evanston, Ill.] *Urol. & Cutan. Rev.*, **48**:511-516. 1944.

The author has made a study of apparently normal kidneys, of minute tumors discovered by chance, and of large advanced neoplasms. On the basis of the theory of prospective potency of cells of the kidney, he concludes that the intrarenal Grawitz tumor develops from renal cortical papillomas that have arisen from proliferation of the lining of renal cortical cysts, and that contain cells capable of forming adrenal cortical tissue. This tumor may be classified as "hybrid" because it exhibits characteristics of two different tissues never found together normally.—V. F. M.

**Wilms' Tumor in a Horseshoe Kidney. Case Report.** ELIASON, E. L., and STEVENS, L. W. [Philadelphia, Pa.] *Ann. Surg.*, **119**:788-790. 1944.

The child recovered after removal, without preoperative irradiation, of the left renal component (containing the tumors). As a protective measure, nephropexy on the opposite side is contemplated for the future.—W. J. B.

**Carcinoma of Renal Pelvis. Histopathologic Study of Seventy-Five Cases with Special Reference to Prognosis.** MCDONALD, J. R., and PRIESTLEY, J. T. [Mayo Clin., Rochester, Minn.] *J. Urol.*, **51**:245-258. 1944.

In the series of 75 cases studied, males predominated over females 3 to 1. Only 4 patients were less than 40 years old. There were 9 instances of calculi. Hydronephrosis was present in 70.7% of the cases; 27 of the tumors were papillary without infiltration, 25 were papillary with infiltration, and 23 were nonpapillary with infiltration. Prognosis was "fairly good" (52% 5 year survivals) for patients with papillary tumors without infiltration, but poor in the other groups. The hilar veins or lymphatics were found involved in 45.3%, but only 1 artery contained tumor; 47.1% of the patients without evidence of vein or lymphatic involvement, and 4.3% of those with such involvement, survived 5 years. The authors specifically avoid using the term "papilloma," since the 5 year survival rate in the most benign types was only 52%. They favor the hypothesis of multicentricity rather than lymphatic spread or transplantation to explain the tumors in the ureter and bladder. The best treatment is nephro-ureterectomy.—V. F. M.

**Tumors of the Ureter.** MCMAHON, S. [Durban, South Africa] *J. Urol.*, **51**:616-622. 1944.

This is an illustrated report of 2 instances of ureteral tumor, one being a villous transitional cell papilloma without signs of bilharzial infestation, and the other, a hard, ovoid, nonneoplastic tumor due to bilharziasis.—V. F. M.

**Primary Sarcoma of the Ureter.** KRAUS, J. E. [St. Francis Hosp., Peoria, Ill.] *Urol. & Cutan. Rev.*, **48**:522-525. 1944.

An instance of this rare lesion is described, with illustrations.—V. F. M.

**Total Cystectomy for Carcinoma.** GRAVES, R. C., and THOMSON, R. S. [Boston, Mass.] *J. Urol.*, **52**:448-454. 1944.

Of 28 patients upon whom the authors have performed cystectomy 13 are alive and free of disease from 1 month to 10½ years after operation. Indications and technic are discussed.—V. F. M.

**Uretero-Intestinal Anastomosis in Two Stages for Cancer of the Bladder: Modification of Original Technique and Report of 33 Cases.** JEWETT, H. J. [Johns Hopkins Hosp., Baltimore, Md.] *J. Urol.*, **52**:536-562. 1944.

This is a review of 33 cases of uretero-intestinal anastomosis performed during the past 4 years by the author's special technic. The lessons learned are discussed. The improvement of the method seems indicated by the occurrence of only 1 death among the last 10 patients, compared to 9 in the first 23.—V. F. M.

**Extensive Glandular Proliferation of the Urinary Bladder Resembling Malignant Neoplasm.** LOWRY, E. C., HAMM, F. C., and BEARD, D. E. *J. Urol.*, **52**:133-138. 1944.

The microscopic examination of biopsy specimens from 3 supposedly malignant bladder neck tumors disclosed benign proliferating glandular tissue covered with normal transitional epithelium. The condition seems closely related to cystitis glandularis.—V. F. M.

**Xanthoma and Carcinoma in a Diverticulum of the Urinary Bladder.** SCHOLL, A. J. [Los Angeles, Calif.] *J. Urol.*, **52**:305-308. 1944.

A case report, illustrated.—V. F. M.

**Papillomatosis of Bladder and Entire Urethra. Infiltrating Cancer of Bladder. Late Pulmonary Metastasis. Successful Pneumonectomy.** DEMING, C. L., and LINDSKOG, G. E. [Yale Univ. Sch. of Med., and New Haven Hosp., New Haven, Conn.] *J. Urol.*, **52**:309-318. 1944.

A male with multiple recurring neoplasms of the bladder and urethra for 13 years developed a lung metastasis. Pneumonectomy has given a satisfactory immediate result. Photomicrographs are included.—V. F. M.

**Primary Carcinoma of the Anterior Male Urethra: Case Report.** HICKEY, R. F., and COLEMAN, R. C., JR. [St. Louis City Hosp., St. Louis, Mo.] *J. Urol.*, **51**:643-645. 1944.

A patient with huge inguinal metastases was found to have the primary lesion in the distal urethra.—V. F. M.

## ORAL CAVITY AND UPPER RESPIRATORY TRACT

**Chemosurgical Treatment of Cancer of the Lip. A Microscopically Controlled Method of Excision.** MOHS, F. E. [State of Wisconsin Gen. Hosp., and McArdle Memorial Lab. for Cancer Research, Madison, Wis.] *Arch. Surg.*, **48**:478-488. 1944.

A fixative paste (40 gm. stibnite of 80 mesh particle size, 10 gm. powdered sanguinaria, and 34.5 cc. saturated solution of zinc chloride) is applied to the cancer in a thickness of less than 1 mm. to 3 mm. or more, and covered with cotton and petrolatum. After 24 hours the first layer of fixed tissue including most of the neoplasm is excised. If cancer is grossly observed in the remaining tissue the fixative is reapplied, and 24 hours later another

layer of tissue is excised and examined microscopically to determine whether excision has been complete. If not, the application of the paste and excision are repeated. Relatively slight deformities are said to be obtained.

Among 176 cases of squamous cell carcinoma of the lower lip treated by this method, 172 primary lesions (97.7%) were completely eradicated. The follow-up period was from 6 months to more than 7 years. No failures were attributed to the method, but treatment was incomplete in 3 cases because of invasion of the mandible and in 1 because of the poor cooperation of a demented patient. Metastases to regional lymph nodes occurred in 20 cases including the 4 last mentioned. Of the remaining 16 patients, only 5 first showed the metastatic lesions after removal of the primary focus (2.8% of 175 patients). Successful results were still evident in 91.5% of 164 cases after 6 months, in 89% of 73 patients after 3 years, and in 87.5% of 32 cases after 5 years. Forty-one patients had been treated unsuccessfully by caustics, radium, x-rays, or surgery alone or combined, and of these, 33 (80.5%) were successfully treated by chemosurgery, as compared to 95.3% success when chemosurgery was applied to those previously untreated.

Eight patients with cancer of the upper lip were treated by chemosurgical means; after 6 months, 5 of 7 patients followed (71.4%) were apparently free from cancer, and at the end of 5 years, 2 of 4 were apparently free.

Though regional metastases were usually removed by standard surgical dissection, large fixed submaxillary masses were removed chemosurgically if the grade of malignancy was not too great and if there were no inoperable lesions in the cervical chain. Among 12 patients with large fixed submaxillary masses treated chemosurgically (after removal of the primary lesion by surgery, irradiation, or chemosurgery), 1 appeared free from cancer after 2 years, and 1 was free from cancer for 1 year and died 3 years later of undetermined cause.—W. A. B.

**Basal Cell Lesions of the Nose, Cheek and Lips.** Davis, W. B. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Ann. Surg.*, **119**:944-948. 1944.

Excision with plastic repair if necessary is the method of treatment preferred in early and moderately advanced basal cell epitheliomas. A prosthesis may be more desirable than attempts at plastic repair when there is pronounced destruction of tissue.—W. J. B.

**Cancer of the Tongue: Its Diagnosis and Treatment.** Jorstad, L. H., and Verda, D. J. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:1077-1088. 1944.

Radium emanation in the form of gold radon seed implanted into the tongue has proved to be the most satisfactory method for controlling cancer of this organ. Adequate radiation averages approximately 200 mgm. hours of radon destroyed per cc. of tissue. Surgical resection of the lymph-bearing tissue in the secondary drainage zone (neck dissection) is the treatment of choice for controlling metastases. Surgery of the tongue itself has not generally given good results. Five-year survival rates were 11% among 51 Barnard patients treated by surgery alone, and 18 and 66%, respectively, among 55 Barnard and 30 private patients treated by radon plus resection of any lymph-bearing tissue. The superior results in the private series are attributed to the greater proportion of early cases.—J. L. M.

**Adenomyoepithelioma (Cylindroma) of Palatal Mucous Glands.** Bauer, W. H., and Fox, R. A. [Sch. of Dentistry, and Sch. of Med., St. Louis Univ., St. Louis, Mo.] *Arch. Path.*, **39**:96-102. 1945.

A morphological study based on 6 cases, with references to the literature. Although this tumor may be remotely related to the "mixed tumors," the authors regard it as a specific type of neoplasm. Although benign, it may undergo cancerous alteration.—J. G. K.

**Treatment of Metastatic Carcinoma of the Neck.** Brown, J. B., and McDowell, F. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Ann. Surg.*, **119**:543-555. 1944.

The opinion is expressed and supported by evidence from the literature that patients with primary carcinoma of the lip or mouth should have dissection of the neck even when there is no enlargement of lymph nodes. Widespread involvement, fixation of metastases to skull or vertebrae, and incurability of the primary lesion are contraindications. Delaying operation is safest in early low-grade neoplasms of the lip when the patient can be followed carefully.

A bibliography of 124 references is appended.—W. J. B.

## Intrathoracic Tumors—Lungs—Heart

**The Modern Successful Treatment of Bronchiogenic Carcinoma.** Graham, E. A. [Washington Univ. Sch. of Med., Barnes Hosp., and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *S. Clin. North America,* **24**:1100-1107. 1944.

Since a limited removal, *i. e.,* by lobectomy, seems to be as little successful in cancer of the lung as the limited removal of portions of other organs affected by cancer, total pneumonectomy has become the commonly accepted therapeutic procedure of choice. Since 1939, the author and his colleagues have performed 83 total pneumonectomies (77 for malignant neoplastic disease), with 23 hospital deaths. The total number of pneumonectomies performed by Blades, Poppe, and Graham during the past 11 years is 123. Five patients are well more than 5 years after this operation for bronchogenic carcinoma, and 1 is well for more than 4 years. Three of these patients had squamous cell carcinoma, and 3 had carcinoma of other types. The periods of survival are as follows: 11 years; 8 years and 5 months; 7 years; 6 years and 8 months; 5 years and 5 months; 4 years and 6 months. The possibility of a permanent cure of bronchogenic carcinoma by the operation of total pneumonectomy seems therefore to be definitely established. The chief problem is early diagnosis and treatment.—J. L. M.

**The Present Status of the Surgical Treatment of Primary Carcinoma of the Lung.** Rienhof, W. F., Jr. [Johns Hopkins Univ. Med. Sch. and Hosp., Baltimore, Md.] *J. A. M. A.*, **126**:1123-1128. 1944.

Proof that total pneumonectomy is not just another surgical experiment but is an operation that has gained a permanent place in our surgical procedures is to be found in the analysis of the results in 71 cases observed

at the Johns Hopkins Hospital. Primary carcinoma of the lung, an otherwise fatal disease, can be satisfactorily treated by total pneumonectomy. The postoperative mortality and longevity are at least as good as, if not better than, the postoperative results following the surgical treatment of carcinoma of other organs. Of the 71 patients undergoing pneumonectomy for primary carcinoma of the lung, 15 died as a result of the operation, 31 died 1 month to 5 years afterward, and 25 were alive and well at the time of writing, 1 month to 11 years after surgery.—M. E. H.

**Alveolar Cell Carcinoma of the Lung.** Ikeda, K. [Charles T. Miller Hosp., St. Paul, Minn., and Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Am. J. Clin Path,* **15**: 50-63. 1945.

An attempt has been made to distinguish a type of pulmonary carcinoma, designated as alveolar cell carcinoma of the lung, from the usual form of bronchogenic carcinoma with which it has heretofore been identified, by discussing its genesis and clinical and histologic characteristics. The accurate incidence of alveolar cell carcinoma of the lung, as such, is now unobtainable, because of the lack of unanimity among the observers regarding its histogenesis. However, it is believed to be higher than generally known. One typical alveolar cell carcinoma of the lung and two others that meet the essential clinicopathologic criteria of this tumor are added to the literature, which claims a total of not more than 50 cases up to the present.—Author's summary. (J. G. K.)

**Mediastinal Ganglioneuroma.** Gray, H. K., Shepard, D. V., and Dockerty, M. B. [Mayo Clin., Rochester, Minn.] *Arch. Surg.,* **48**:208-213. 1944.

Discussion and report of 3 cases.—W. A. B.

**Clinicopathologic Conferences. Case 1. Von Recklinghausen's Neurofibromatosis with Neurofibrosarcoma of the Mediastinum. Case 2. Right Thoracic Sarcoma.** Wakefield, H., and Hirsch, E. F. [St. Luke's Hosp., Chicago, Ill.] *Illinois M. J.,* **87**:49-52. 1945.

The clinical history and conditions demonstrated by postmortem examination in Case 1 are characteristic of systemic neurofibromatosis in which an intrathoracic growth progressed into sarcoma. The growth of sarcoma in the tissues of the right side of the thorax in Case 2 is considered to be independent of the fibroadenoma of the breast removed 7 years before. The chief reasons for this opinion are the absence of recurrence at the site of removal and the extensive diffuse growth of the tumor tissues in the parietal and visceral tissues of the right side of the chest.—M. E. H.

**Hemangioma of the Mediastinum.** Adams, W. E., and Bloch, R. G. [Univ. of Chicago, Chicago, Ill.] *Arch. Surg.,* **48**:126-129. 1944.

Report of a case.—W. A. B.

**Mediastinal Teratoma.** Wilson, S. J., and Cares, R. *Arch. Path.,* **39**:113-116. 1945.

Report of a case in which a solid teratoma was found in a man aged 29. The growth contained choriocarcinomatous elements and had metastasized to a lymph node in the neck.—J. G. K.

**Cysts and Cystic Tumors of the Mediastinum.** Laipply, T. C. [West. Reserve Univ., and Univ. Hosps., Cleveland, Ohio] *Arch. Path.,* **39**:153-161. 1945.

Classification and discussion based on an extensive review of the literature and case reports (1 each) of dermoid, bronchial, and gastric cysts of the mediastinum. About 11% of the epidermoids, dermoids, and teratomas reported in the literature were cancerous; all other types of congenital mediastinal cysts were noncancerous.—J. G. K.

**Primary Tumor of the Heart.** Straus, R., and Merliss, R. [Cedars of Lebanon Hosp., Los Angeles, Calif.] *Arch. Path.,* **39**:74-78. 1945.

Three cases of primary tumor of the heart are reported, of which 2 were myxomas and 1 was a myxosarcoma.—J. G. K.

**Congenital Glycogenic Tumors of the Heart.** Batchelor, T. M., and Maun, M. E. [Wayne Univ. Coll. of Med., and St. Mary's Hosp., Detroit, Mich.] *Arch. Path.,* **39**: 67-73. 1945.

Report of a case of multiple tumors of the heart in a 3 day old boy, in which glycogen was demonstrated in the tumor cells. A review of the literature on so-called congenital rhabdomyoma of the heart disclosed reports of 62 cases of tumors of the heart that presented a characteristic vacuolated appearance on microscopic examination. The authors suggest that the term "congenital nodular glycogenic tumors of the heart" should replace "congenital rhabdomyoma" until more is known about the nature of the lesion.—J. G. K.

## Gastrointestinal Tract

**Esophageal Carcinoma in British West Indian and Panamanian Negroes. A Study of the Incidence, Etiologic Factors and Pathologic Anatomy in Fifty Cases.** Tomlinson, W. J., and Wilson, L. A., Jr. *Arch Path.,* **39**:79-80. 1945.

Twenty-six of the 50 persons with esophageal carcinoma presented clinical and autopsy evidence of syphilis, while the average incidence of syphilis in the entire autopsy population during the past 10 years was 8.7%. —J. G. K.

**Carcinoma of the Oesophagus with Tracheal Fistula and Tuberculous Bronchopneumonia. Clinicopathologic Conferences St. Luke's Hospital.** Wakefield, H., and Hirsch, E. F. [St. Luke's Hosp., Chicago, Ill.] *Illinois M. J.,* **87**:104-106. 1945.

A case of squamous cell carcinoma of the esophagus in a 64 year old man was unsuccessfully treated by x-ray and terminated fatally. The cancerous growth caused a tracheal fistula, and the course of the disease was complicated by an extensive tuberculous bronchopneumonia. —M. E. H.

**Lymphosarcoma of the Gastro-Intestinal Tract. Report of Twenty Cases.** McSwain, B., and Beal, J. M. [New York Hosp., and Cornell Univ. Med. Coll., New York, N. Y.] *Ann. Surg.,* **119**:108-123. 1944.

In a series of 13 males and 7 females, lymphosarcoma was found distributed throughout the gastrointestinal tract. Besides large and small cell lymphosarcomas there were 3 giant follicle sarcomas and 1 recticulum cell sar-

coma. In 3 cases the diagnosis was made from the barium series. Lesions of large size with symptoms of short duration and whorl-like defects in the films are suggested as diagnostic points in favor of lymphosarcoma. Nine patients are now living from 1 to more than 9 years after diagnosis. Resection was followed by survival for from 2 to 7 years in 6 patients operated on (1 died later of an unrelated disease); 2 of 6 patients treated with x-ray only are without recurrence. In 2 instances, after surgery and x-ray combined, there is likewise no evidence of recurrence.—W. J. B.

**An Experiment in the Early Diagnosis of Gastric Carcinoma.** St. John, F. B., Swenson, P. C., and Harvey, H. D. [Coll. of Physicians and Surgeons, Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *Ann. Surg.*, **119**:225-231. 1944.

Rapid fluoroscopic examination of the stomach was made on 2,413 persons in whom gastric lesions were unsuspected, and examination was repeated on 491 after a year or more. Two had carcinomas, which were resected. One individual had lymphosarcoma, which was found only after a second operation. This patient later died. Resection was carried out in 5 cases proving to be benign ulcer, and in 1 of these a pulmonary embolus caused death. Abnormalities other than neoplasia were found 528 times. The authors are conservative in their viewpoint concerning the practical application of mass roentgenography, but feel that further study is desirable in view of the poor outlook at present for cure in patients with carcinoma of the stomach.—W. J. B.

**Total Gastrectomy, Splenectomy, Resection of the Left Lobe of the Liver, Omentumectomy and Colectomy upon One Patient in One Operation.** Lahey, F. H. [Lahey Clin., Boston, Mass.] *Ann. Surg.*, **119**: 222-224. 1944.

The patient was thought at the time of operation to have an invasive carcinoma of the stomach. Five months later he died with Hodgkin's disease.—W. J. B.

**Neurofibroma of the Small Intestine. Report of Case.** Collins, J. D. [Portsmouth, Va.] *Ann. Surg.*, **119**: 362-371. 1944.

Eighteen cases from the literature, and 1 original case of neurofibroma with volvulus and intestinal obstruction, are discussed.—W. J. B.

**Carcinoma of the Duodenum. Report of Two Cases in Suprapapillary Portion.** Cohn, I. [New Orleans, La.] *Ann. Surg.*, **119**:342-350. 1944.

Although the results in these cases are not encouraging, they are presented in order to emphasize the point that lesions of the duodenum may be neoplastic. If this is kept in mind, it may more often be possible to make the diagnosis preoperatively, as was done in these cases.—W. J. B.

**Pancreaticoduodenectomy for Primary Carcinoma of the Duodenum.** Orr, T. G., and Walker, G. A. [Univ. of Kansas Sch. of Med., Kansas City, Kans.] *Surg., Gynec. & Obst.*, **80**:149-151. 1945.

Case report and discussion.—J. G. K.

**Multiple Adenomas of the Jejunum. Case Report.** Dickson, W. B. [Slocum-Dickson Clin., Utica, N. Y.] *Ann. Surg.*, **119**:283-286. 1944.

Some of the adenomatous polypi were removed at 2 operations. The patient has been followed for 11 years. A malignant granulosa-cell tumor of the ovary also was removed at the second operation, and metastases from it have responded to x-ray.—W. J. B.

**Neurogenic Sarcoma of the Jejunum Associated with von Recklinghausen's Disease.** Hamilton, J. B., Kennedy, P. C., and Herault, P. C. [Atlanta, Ga.] *Ann. Surg.*, **119**:856-864. 1944.

The patient had intestinal bleeding and secondary anemia. The lesion in the jejunum was resected successfully. According to the literature, neurogenic sarcoma occurs frequently in conjunction with von Recklinghausen's disease, but rarely in the jejunum.—W. J. B.

**Enterogenous Cysts at the Ileocecal Junction.** Rosenburg, S. A. [Montefiore Hosp., Pittsburgh, Pa.] *Ann. Surg.*, **119**:873-877. 1944.

This condition should, in the author's opinion, be treated by marsupialization, rather than by resection and anastomosis of the intestine or by evacuation or enucleation of the cyst. A case treated by marsupialization followed by the use of escharotics is presented.—W. J. B.

**Obstructive Submucous Lipoma of the Cecum.** Moore, E. C. [Moore-White Clin., Los Angeles, Calif.] *California & West. Med.*, **60**:21-24. 1944.

A report of a case, and a discussion of differential diagnosis and therapy. If the nature of the tumor is recognized at surgery and if the patient is in exceptionally good general condition, the growth may be resected after simple enterostomy, without the intestinal mucosa being opened. If complete intestinal obstruction and other factors make the patient a poor risk, a 2-stage procedure is advisable.—M. H. P.

**Pregnancy Associated with Carcinoma of the Large Intestine.** Banner, E. A., Hunt, A. B., and Dixon, C. F. [Mayo Foundation, and Mayo Clin., Rochester, Minn.] *Surg., Gynec. & Obst.*, **80**:211-216. 1945.

A report of 7 cases, with a review of the literature and discussion. The maternal and corrected fetal mortality rates were both 14.3% in the authors' series.—J. G. K.

**Multiple Carcinomas of the Large Intestine.** Berson, H. L., and Berger, L. [Jewish Hosp., Brooklyn, N. Y.] *Surg., Gynec. & Obst.*, **80**:75-84. 1945.

Sixty-six cases of 2 cancers of the large intestine, and 6 cases of 3 cancers of this organ, were collected from the literature since 1932. To these are added 13 patients with 2, and 3 patients with 3 primary cancers. The frequency of multiple cancers was found to be 4.6% in a series of 344 cases of carcinoma of the large bowel. Polyps were present in 25% of patients with a single cancer and in 37.5% of those with multiple carcinomas.

If 1 cancer of the large intestine is found during diagnostic procedures or surgery, a careful search should be made for others.—J. G. K.

## Peritoneum

**About a Reliable Symptom of Extrauterine Endometriosis.** Forti, E. *Exper. Med. & Surg.*, **2**:64-69. 1944.

Fainting spells before menstrual periods were reported by several patients later found at operation to have endoperitoneal endometriosis. Such spells, attributed to peritoneal irritation by blood from desquamating endo-

metrial tissue, are regarded by the author as a diagnostic sign of endometriosis. In the 2 illustrative cases, the endometriosis was located on the peritoneal surface of Douglas' pouch, and on a portion of the ovary adherent to the posterior peritoneum, respectively. Photomicrographs.—M. H. P.

### Muscle

**Primary Hemangiomatous Tumors of Skeletal Muscle.** Shallow, T. A., Eger, S. A., and Wagner, F. B., Jr. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Ann. Surg.*, **119**: 700-740. 1944.

The authors review 335 cases from the literature, and present 2 cases of their own in which the hemangioma was excised.—W. J. B.

**Rhabdomyosarcoma of the Extremities.** Garber, R. L. [Good Samaritan Hosp., Cincinnati, Ohio] *Radiology*, **42**: 595-596. 1944.

A case report.—R. E. S.

### Leukemia, Lymphosarcoma, Hodgkin's Disease

**Myelogenous Leucemia Complicating Pregnancy.** Angelucci, H. M. [Woman's Hosp., and Woman's Med. Coll. of Pennsylvania, Philadelphia, Pa.] *Am. J. Obst. & Gynec.*, **48**: 125-128. 1944.

A pregnant woman showed aggravation of chronic myelogenous leukemia as pregnancy advanced, and benefited from x-ray after potassium arsenite medication became inadequate. She was delivered of an apparently normal child.—A. K.

**Spontaneous Rupture of the Spleen in Chronic Myeloid Leukemia.** Greenfield, M. M., and Lund, H. [Cleveland, Ohio] *Ohio State M. J.*, **40**:950-951. 1944.

Rupture of the spleen apparently followed infarction in the enlarged organ. The patient lived about 2 days after showing signs of sudden peripheral vascular collapse.—E. E. S.

**Leukemia as a Cause of Nasal Obstruction.** Hatch, F. N. [Laguna Honda Home, San Francisco, Calif.] *California & West. Med.*, **61**:23-26. 1944.

A 61 year old woman with monocytic leukemia developed infiltrations of the nasal mucosa leading to obstruction. Local x-ray therapy failed in this instance, but is recommended for trial in future cases. The literature on nasal complications of leukemia is reviewed with bibliography.—M. H. P.

**Hodgkin's Disease of the Breast.** Adair, F. S., Craver, L. F., and Herrmann, J. B. [Memorial Hosp., New York, N. Y.] *Surg., Gynec. & Obst.*, **80**:205-210. 1945.

Review of the literature with the addition of 5 cases.—J. G. K.

### Pancreas

**Total Pancreatectomy for Hyperinsulinism Due to an Islet-Cell Adenoma. Survival and Cure at Sixteen Months after Operation. Presentation of Metabolic Studies.** Priestley, J. T., Comfort, M. W., and Radcliffe, J., Jr. [Mayo Clin., Rochester, Minn.] *Ann. Surg.*, **119**:211-221. 1944.

This case is reported as the first with survival beyond the immediate postoperative period after such a procedure. Mild diabetes and diminution in digestion of fat and protein followed the operation.—W. J. B.

**Adenomatosis of Islet Cells, with Hyperinsulinism.** Frantz, V. K. [Coll. of Physicians and Surgeons, Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *Ann. Surg.*, **119**:824-844. 1944.

Cases from the literature and from the author's series are brought up to date. Tumor was found in 27 of 32 patients explored after a diagnosis of hyperinsulinism had been made. Two cases of adenomatosis, with improvement following partial pancreatectomy, are presented in detail. In the cases with and without proved malignancy, 20% and 14.2% of the tumors respectively were multiple, suggesting multicentric origin of tumors of the islet cell. A 5-page bibliography is included.—W. J. B.

**Adenocarcinoma of the Head of the Pancreas.** Thompson, R. T., and Abe, G. Y. [Cincinnati Gen. Hosp., Cincinnati, Ohio] *Ohio State M. J.*, **41**:46-48. 1945.

A case report. The tumor obstructed bile and pancreatic ducts at the ampulla of Vater, and was accompanied by multiple liver abscesses and septicemia. Prompt surgery is urged in instances of tumors at the ampulla of Vater.—E. E. S.

**Carcinoma of Head of Pancreas and Ampulla of Vater.** Mullen, B. P. [Seattle, Wash.] *Northwest Med.*, **44**: 14-18. 1945.

Two case reports. Both patients had resection of the tumor. One succumbed 3 months later, perhaps from an infarct of the heart. The other survived and was well 3½ months after operation. Variations of the operative technic are discussed.—E. E. S.

### Thyroid

**Thyroid Carcinoma with Metastases: Studied with Radioactive Iodine.** Frantz, V. K., Ball, R. P., Keston, A. S., and Palmer, W. M. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Ann. Surg.*, **119**: 668-689. 1944.

A patient, who survived 16 years after a thyroid carcinoma was first noted, died 9 years after excision of the primary mass and approximately 5 years after metastases were evident clinically. Serum cholesterol values and basal metabolic rate were normal at every determination. In only one metastatic focus (in the femur) was a consistent uptake of radioactive iodine proved. It was the only metastasis with gross appearance of thyroid tissue; microscopically most of the tissue from this focus was well differentiated. The therapeutic use of radioactive iodine is suggested for control of small, well differentiated metastases from thyroid carcinoma.—W. J. B.

### Miscellaneous

**Fibrosarcoma of the Extremities.** Carroll, G. A., and Martin, T. M. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *S. Clin. North America*, **24**:1220-1227. 1944.

This is a survey of the fibrosarcomas of the extremities (excluding those limited to the skin) that have been seen at the Barnard Free Skin and Cancer Hospital during the 30 year period from 1915 to 1944. These fibrosarcomas constitute a relatively small group compared to the malignant neoplasms of epithelial origin. They are, how-

ever, important because of their strong tendency to recur locally, and eventually to initiate distant metastases with fatal termination. In 7 of 35 surgically treated cases the patients have survived for 5 or more years. Six additional patients have been followed and are alive and well for 21, 24, 26, 31, 36, and 38 months postoperatively. Thirteen are known to be dead of the disease; 2 have died from other causes less than 5 years after operation; 1 is known to have had a recurrence after 3 years. Radiation has been ineffective. Tumors of long duration before treatment seem to afford a better prognosis than do those of short duration. The average duration of life after treatment in 13 fatal cases was about 8 months.—J. L. M.

**Liposarcoma, the Malignant Tumor of Lipoblasts.** Stout, A. P. [Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *Ann. Surg.*, **119**:86-107. 1944.

Forty-one cases of liposarcoma are reported with a discussion of 134 cases from the literature. The tumors are large, rather firmer than simple lipomas, and of cream to orange color; some are mucoid. It is felt that the several types of liposarcoma originate from the same mesenchymal cell. The myxoid types may be well differentiated and relatively benign, resembling embryonal fat, or poorly differentiated and malignant. The adenoid type with rounded lipoblasts having the appearance of brown fat, and the mixed group complete the classification. Other histological elements are seen as the result of metaplasia. There are reports of liposarcomas at most ages, but the majority occur late in life. Most frequently they are found in the thigh and extraperitoneal tissues. There is wide variation in growth rate; multiple tumors occur; metastases are usually in lungs or liver.

Emphasis is placed on diagnosis of the tumor before treatment is attempted. Radical surgery offers the best hope for cure. Radiotherapy for recurrent nodules was successful in 2 of 8 cases.—W. J. B.

**Mycotic Granulomas Simulating Malignant Disease.** Moore, M. [Barnard Free Skin and Cancer Hosp., Barnes Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**:1044-1063. 1944.

There is an ever increasing number of reported cases of fungous infections, particularly those that are considered to be of the granulomatous type. These mycoses must be differentiated from malignant disease. The better known fungous infections of the skin, lungs, and other organs are listed with a discussion of their clinical manifestations, ways and means of diagnosis, and diseases that they mimic, including oral and pulmonary carcinoma, tumors of brain and spinal cord, leukemia, lymphadenoma, lymphosarcoma, and Hodgkin's disease.—J. L. M.

**Carcinoma in Routine Naval Practice.** Deaver, J. M., and Cronkite, E. P. *U. S. Nav. M. Bull.*, **42**:421-423. 1944.

One case of carcinoma of the rectum, one of adenocarcinoma of the colon, and one of adenocarcinoma of the breast occurred in men of the younger age group.—C. W.

**Report of Cases from St. Mary's Hospital Tumor Clinic.** Wright, C. B., Werthammer, S., and Genge, C. D. [Huntington, W. Va.] *West Virginia M. J.*, **40**:327-329. 1944.

One hundred and two cases of tumors and precancerous lesions are tabulated with respect to the various organs affected. Methods of therapy are mentioned briefly.—E. E. S.

**Newark Beth Israel Hospital Tumor Conference, April 5, 1944.** Singer, M., Chairman. [Newark, N. J.] *J. M. Soc. New Jersey*, **41**:347-350. 1944.

Presentation of cases: Levinson, L. J. Acute wound cancer of the nose; Huberman, J. Combined surgical and radiation treatment of cancer of the ovary; Comando, H. Carcinoma of thyroid; Goldberg, H. Unusual case of Hodgkin's disease; Rados. Reticulum cell sarcoma of the eyelids.—M. E. H.

**Case Reports of Barnes Hospital. Clinical and Postmortem Records Used in Weekly Clinicopathologic Conferences at Barnes Hospital, St. Louis.** Wood, W. B., Jr., and Moore, R. A., Editors. [St. Louis, Mo.] *J. Missouri M. A.*, **41**:206-212. 1944.

Two case reports. Case 53 proved to be an adenocarcinoma of the prepyloric part of the stomach with partial stenosis of the pylorus, and case 54, an undifferentiated carcinoma of the right lung with metastases.—M. E. H.

**Neurofibroma Within a Lipoma of the Neck.** Hallson, C. H., and Marcuse, P. [Jefferson Davis Hosp., Houston, Tex.] *Am. J. Surg.*, **64**:268-270. 1944.

A case report.—W. A. B.

**A Paraumbilical Abdominal Wall Cyst.** Kidd, F. H., Jr. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Ann. Surg.*, **119**:279-282. 1944.

A case report and discussion of the origin of the cyst.—W. J. B.

**Presacral Dermoid. Case Report.** Carney, H. M. [300th Gen. Hosp., Camp Forrest, Tenn.] *Ann. Surg.*, **119**:155-158. 1944.

A very large cyst was dissected free after the coccyx had been removed.—W. J. B.

**A Consideration of Certain Radical Procedures in the Treatment of Cancer.** Pack, G. T. [Memorial Hosp., New York, N. Y.] *West Virginia M. J.*, **41**:65-71. 1945.

Three radical operations are discussed. The indications for, and advantages, results, and technic of the interscapulothoracic amputation for malignant tumors of the upper extremities are presented in detail. Radical groin dissection for metastatic tumors is advocated in preference to superficial dissection despite the higher mortality, since the incidence of recurrence is high with the latter procedure. The discussion of total gastrectomy for carcinoma of the stomach is largely concerned with technic. Statistics and diagrams are presented.—E. E. S.

**Eponyms in the History of Cancer.** Gilbert, J. B. [Schenectady, N. Y.] *New York State J. Med.*, **44**:511-518. 1944.

The author's purpose is to list in approximate chronologic order those workers whose names are attached to classic or epoch-making descriptions in the long history of cancer literature.—J. L. M.

## STATISTICS

**Cancer Statistics for Morticians and for Clinicians.** Cramer, W. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**:1255-1268. 1944.

This is a general discussion of various phases of human

cancer statistics. The use of *numbers* of cases (or deaths), whether in a hospital, city or state, as a basis for analysis, without reference to the population at risk, Cramer calls "undertaker statistics;" the fallacies that may result are pointed out. Failure to allow for age distribution of two populations is another frequent error. Inaccuracies in diagnosis usually result in *under-diagnosis,* but do not necessarily affect differences found between various population groups, which may be well described by human mortality statistics. The practical value of clinical data in eliciting facts concerning occupational cancer, precancerous conditions, sex differences, relation of cancer to other diseases, and familial cancer is stressed. Cramer suggests that a feature of mortality from cancer of the skin, esophagus, and intestine in recent years may be an increase at older ages accompanied by decrease at younger ages (under 65), which, if interpreted as postponement of onset, may be considered a form of control.—M. L. L.

CANCER CONTROL AND PUBLIC HEALTH

**The Place of the National Cancer Institute in the Cancer Program.** SPENCER, R. R. [Nat. Cancer Inst., Bethesda, Md.] *Radiology,* **42**:493-498. 1944.

The role of the National Cancer Institute in cancer education and research is discussed.—R. E. S.

**Pilot Teaching Courses in Cancer.** *Science,* **101**: 425-426. 1945.

Report of a meeting of the National Advisory Cancer Council [U. S. Public Health Service], in which it was proposed that "pilot teaching courses" in cancer be established for third and fourth year medical students for the principal purpose of fitting them to engage in cancer control as future general practitioners.

Among other items it was also proposed that research programs in the future should be guaranteed support for 5 to 10 years instead of for 1 year at a time as has been done in the past.—R. B.

**The Position of the American Cancer Society and the Field Army in Connecticut.** CREADICK, A. N. [New Haven, Conn.] *Connecticut M. J.,* **8**:612-614. 1944.

A general résumé of education for cancer control in the state.—M. E. H.

**Why Worry About Cancer.** ZIMMERER, E. G. [Iowa State Dept. of Health, Des Moines, Iowa] *J. Iowa M. Soc.,* **34**: 417-418. 1944.

The article is directed toward quieting the layman's apprehension over the increasing death rate from cancer throughout the nation. However, seeking early medical advice is urged.—M. E. H.

**Cancer Control, A Doctor's Program.** ZIMMERER, E. G. [Iowa State Dept. of Health, Des Moines, Iowa] *J. Iowa M. Soc.,* **35**:41-44. 1945.

The program of cancer control is and must be a doctor's program.—M. E. H.

**Cancer as a Public Health Problem.** SMITH, H. G. [Bournemouth, England] *Pub. Health,* **57**:56-57. 1944.

A report of the progress of the cancer clinic established by Bournemouth County, England, the first locality to obtain the approval of the Ministry of Health for its program based on the Cancer Act of 1939. Suggestions are made concerning material to be presented in addresses to the public on the subject of cancer.—E. E. S.

**British Empire Cancer Campaign.** *Twenty-first Annual Report.* 1944.

The sum of £71,671 was collected in the year 1943 to 1944, of which £33,180 was used in grants made for research to 15 institutions and £7,325 allotted to 10 Branches for local work. Results achieved are summarized, in a preface by J. P. Lockhart-Mummery, under the headings: Carcinogenic Agents; Chemotherapy; Filterable Agents and Viruses; Pathology; Radiobiology; Radiotherapy; Statistics.—M. H. P.

**Discussion on Post-War Organization for the Treatment of Cancer.** CARLING, E. R., ET AL. *Proc. Roy. Soc. Med.,* **38**:147-154. 1945.

CARLING, E. R. Radiation is, for the most part, conducted by whole-time staff; surgery, very largely by part-time. Whilst radiation is on the whole concentrated in comparatively few places, surgery is distributed over many. One set of circumstances lends itself to a simple and tidy administrative job; the other presents many difficulties. Radiotherapy should be concentrated in a few fully equipped and strongly staffed institutions, each perhaps with a very small group of associated subcenters. The headquarters should be in a university city. A single radiotherapeutic organization can deal with a population up to three and a half or four millions; two millions form a satisfactory number; sparsity of population makes it necessary to provide for an area with as little as a million as a minimum. For *all* hospital purposes the country, outside the London area, can be divided suitably into about a dozen major areas, each with a university center as its natural headquarters.

The Cancer Act provides for those suffering from *or suspected of* cancer. The suspicion of cancer arises in the ambit of the general practitioner. Preliminary investigation centers should therefore be within reasonable immediacy of the patients' homes. Although the flow of patients should be from the periphery towards the center, with careful filtration *en route,* so that the autonomous sufficiency of the intermediate hospital is not neglected, the traffic should be two-way—that is to say, the most expert headquarters help should be at the service of the "district" associates—and that of *their* staff at the service of the general practitioner.

Operation for cancer of a given region of the body ought to be in the hands of those whose daily practice is in that region. Major surgery should not in future be done in institutions where there is no resident; where the pathological and radiological services are second-rate; where collateral specialists cannot be met at will.

The radiotherapist ought to be consulted about every case. This brings us to the question "who should do radium implantation?" Whoever implants ought to have such knowledge of the fields induced by complicated sources as will enable him to secure homogeneous irradiation; he must submit the field he has in fact produced to analysis by a physicist, and be ready to correct it; he must cooperate with the radiotherapist in supplementary

irradiation from external sources. The gynecologist who has completely familiarized himself with the physical basis of irradiation and who, like the radiotherapist, will submit his given case to scrutiny by the physicist, is entitled to carry out insertion of appliances.

STEBBING, G. F. In the United Kingdom in 1942, the following deaths were due to cancers: 7,887 breast; 5,097 uterus; 1,206 larynx; 1,033 tongue; and 290 lip. This state of affairs exists because we have failed to create an organization that will make the best use of the abilities we possess, the instruments we have devised, and the resources we control. The Cancer Act, 1939, recognized the necessity for nation-wide coordination of effort, but so far, in spite of the efforts of the Radium Commission, little has been accomplished. Many doctors working under the National Health Insurance scheme, and particularly in large towns, have to see so many patients in a short time that they cannot find time for the careful consideration of obscure symptoms. Radiotherapy plays an important part in the treatment of cancer, and in many hospitals there is neither an efficient radiotherapy department, nor an established arrangement for obtaining radiotherapy elsewhere. We must have available in every region a cancer organization that is known to every medical practitioner, that is freely and immediately at the disposal of every patient, and that is so arranged that it ensures efficient diagnosis and prompt treatment for every patient. There should be a regional organization with a whole-time director that should serve the needs of a population of about two million people. The headquarters of the organization should be in a general hospital where there is a highly organized radiotherapy department, and where physicians, surgeons, gynecologists, radiodiagnosticians, pathologists, and physicists can consult frequently, and have time and opportunity to discuss the problems of the work in which they are engaged.

Last year there were 1,142 people in Great Britain who died from cancer of the skin, and every radiotherapist knows that if a case of cancer of the skin is taken early the patient does not die.

NUTTALL, J. R. Approximately 45% of patients are beyond any curative treatment when they first seek the advice of the specialist, owing to ignorance and fear on their part, and, to some extent, lack of knowledge of the disease amongst general practitioners. Every million of population requires the provision of a number of diagnostic clinics, and facilities for the treatment of some 600 surgical, and 700 radiotherapeutic cases per year, requiring approximately one hundred hospital beds for active treatment alone. It is generally recognized, however, that to be efficient and economical, cancer-treating units should serve a population greater than one million—two to three millions being considered more satisfactory. A radiotherapy center should serve a population of at least two millions.

CADE, S. In the proper organization of cancer centers in the future the clinical control should be in the hands of a team and not of one director, be he a surgeon or a radiotherapist. Radiotherapists should be trained to use both radium and x-rays and not x-rays only as is so often the case at present to the detriment of patients. Should a national cancer service be realized, cancer ought to be a notifiable disease, in the same way that tuberculosis is.—E. L. K.